Metabolic abnormalities in multiple symmetric lipomatosis: elevated lipoprotein lipase activity in adipose tissue with hyperalphalipoproteinemia

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Abstract Lipoprotein lipase activity in lipomatous tissue, post-heparin lipoprotein lipase activity in plasma, and the composition and concentration of serum lipoproteins were studied in 15 patients with Multiple Symmetric Lipomatosis (MSL). Extremely elevated lipoprotein lipase activity in adipose tissue was found in MSL patients. Total and hepatic post-heparin plasma lipolytic activity was normal, while a moderate but statistically significant increase of extrahepatic lipolytic activity was present. An abnormal composition of serum lipoproteins, characterized by a significant increase in high density lipoproteins, namely HDL_2 subfraction, and apoprotein A-I, was demonstrated. A concomitant decrease in and abnormal composition of low density lipoproteins were found. This lipoprotein pattern is consistent with a newly recognized type of hyperalphalipoproteinemia. Significant correlations were found between serum HDL_2 cholesterol values and lipoprotein lipase activity in adipose tissue (as well as between serum VLDL-triglyceride and HDL_2 cholesterol values). These observations confirm the role of adipose tissue lipoprotein lipase in triglyceride-rich lipoprotein catabolism. The elevated levels of lipoprotein lipase activity in adipose tissue, in addition to a previously demonstrated decrease in adrenergic-stimulated lipid mobilization, could account for both the abnormal fat accumulation in lipomatous fat cell and for hyperalphalipoproteinemia in MSL patients. The occurrence of MSL in two brothers suggests an inherited enzymatic defect, indicating MSL as a "triglyceride storage disease in adipose tissue".—**Enzi, G., L. Favaretto, S. Martini, R. Fellin, A. Baritussio, G. Baggio, and G. Crepaldi.** Metabolic abnormalities in multiple symmetric lipomatosis: elevated lipoprotein lipase activity in adipose tissue with hyperalphalipoproteinemia. *J. Lipid Res.* 1983. **24:** 566–574.

Supplementary key words post-heparin plasma lipolytic activity • apoproteins • VLDL • HDL_2

The presence of slowly growing, symmetrically disposed fat masses at the neck, the shoulders, the deltoid region, the trunk, and groin characterizes the outward feature of patients with multiple symmetric lipomatosis (MSL) (**Fig. 1**).

Different metabolic abnormalities, such as reduced glucose tolerance, hyperuricemia, hyperlipemia, or tubular renal acidosis have been reported to be associated with MSL (1–7), none of them with pathognomonic metabolic features.

In recent years a specific defect in adrenergic-stimulated lipolysis in lipomatous tissue has been demonstrated (8). Further studies confirmed this finding even though the precise site of the regulatory defect has not been defined yet (9, 10). Thus, multiple or differently located defects in the lipolytic cascade have been hypothesized (9). Moreover, isolated observations of increased lipoprotein lipase (LPL) activity in lipomatous tissue from MSL have been reported (11, 12).

The aim of this study is to evaluate possible alterations in LPL activities and in circulating lipoprotein composition in a group of MSL patients large enough to provide definitive information.

PATIENTS AND METHODS

Fifteen male patients from northeastern Italy aged 34–65 years and affected by MSL were studied during a short hospital stay. The duration of the disease ranged between 2 and 18 years. Alcohol intake, obtained by a 7-day recall, ranged between 60 and 220 ml per day. Individual data on daily alcohol intake are reported in Table I. The metabolic studies were carried out after an overnight fast without dietary preparation. Body fat mass was calculated according to the Edwards and White formula (13), using height and body weight as parameters. All subjects gave their informed consent

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Abbreviations: MSL, multiple symmetric lipomatosis; LPL, lipoprotein lipase; PHLA, post-heparin lipolytic activity; FFA, free fatty acid; HSL, hormone-sensitive lipase.
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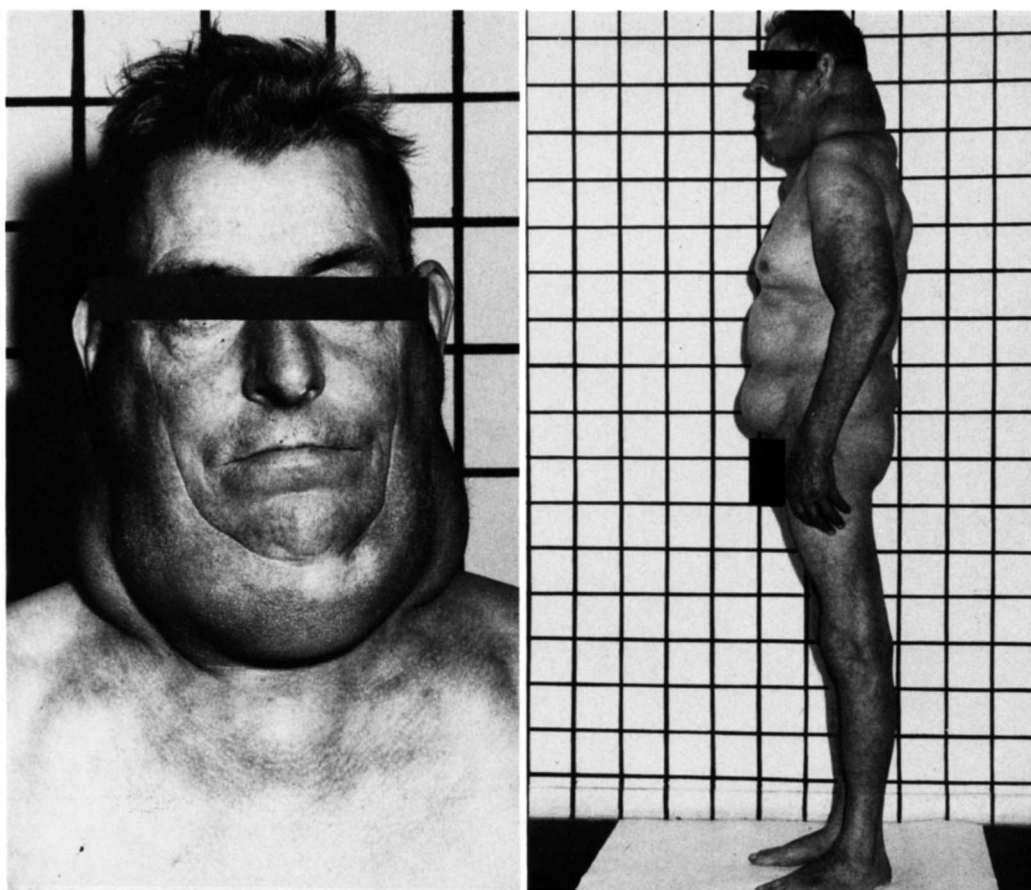
Fig. 1. A patient with MSL.

for the study, which was approved by the ethical committee of the Medical Faculty.

Studies on lipoprotein lipase activity

LPL activity in adipose tissue was measured in ten MSL patients on 100–150-mg lipomatous tissue samples obtained by percutaneous needle biopsy from the deltoid region. Ten metabolically normal, slightly overweight male subjects, matched for age, volunteered for fat biopsies (control group). The samples were immediately transferred to the laboratory for fat cell sizing and, according to Pykälisto, Smith, and Brunzell (14), modified as follows for LPL determination. Incubation for LPL elution was carried out in triplicate (20–30 mg of tissue per ml of medium), for 40 min, with gentle shaking, at 37C°. The medium was Krebs-Ringer-Tris buffer, pH 8.0, plus 1% fat-free bovine albumin fraction V, 10% human fasting serum, and 5 mg/100 ml of heparin sodium. At the end of the incubation period, 0.1 ml of medium containing the LPL eluate was added to 0.1 ml of a substrate constituted by glycerol-tri (1-^{14}C)oleate, 2 μCi/ml and unlabeled triolein, 3 μmol/ml

in 1 ml of 5% arabic gum solution in 0.2 M Tris-HCl, pH 8.0. The substrate mixture was emulsified by sonication for 3 min at maximum power (A 350 G Ultrasonic Ltd. sonifier) in ice-cooled vials. Duplicate samples of eluate-substrate mixture were incubated at 37C°, with continuous gentle shaking for 60, 90, 120 min. As a blank, 0.1 ml of substrate was added to 0.1 ml of incubation medium without eluate. At the end of each period of incubation, 3.25 ml of methanol–chloroform–N-heptane 1.41:1.25:1 (v/v) was added to the samples for oleate separation (15). After shaking, 1.05 ml of a solution containing 50 mM K_2CO_3, 50 mM KOH, and 50 mM $K_2B_4O_7$–4 H_2O, pH 10, was added. After vigorous shaking, the samples were centrifuged at 1200 rpm for 15 min at room temperature; 0.5 ml of supernatant was then transferred to counting vials (scintillation liquid: toluene POP-POPOP in methanol). The recovery of the oleate was determined in each experiment: 100 μl of (1-^{14}C)oleic acid was added to 100 μl of unlabeled oleic acid and then treated as the samples ("recovery"); simultaneously 100 μl of (1-^{14}C)oleic acid in 1 ml of toluene was directly added to the scintillation

TABLE 1. Anthropometric and routine metabolic parameters in MSL patients

Patient	Age	Height	Body Weight	Body Mass Index	Body Fat Mass	Alcohol Intake	Blood Glucose	Cholesterol	HDL Chol.	Triglycerides	Phospholipids	LP electrophoresis[a]
	yr	cm	kg	W/H²	kg	ml/day			mg/dl			
1) Za.A.	34	162	50.1	19.1	5.4	100	65	217	93	76	217	HyperalphaLP
2) Ma.G.	51	160	58.0	20.7	7.5	120	108	155	68	50	198	Normal
3) Ma.D.	48	160	53.0	19.5	7.5	60	80	187	76	706	312	V
4) Ve.A.	49	164	66.0	23.1	12.2	120	97	279	109	148	376	HyperalphaLP
5) Io.A.	40	168	74.6	25.4	18.2	200	82	248	103	199	388	IV HyperalphaLP
6) Pa.D.	63	164	91.2	33.9	36.0	240	95	202	73	223	340	IV HyperalphaLP
7) D.N.O.	55	162	51.0	19.5	5.7	120	95	231	67	176	302	Normal
8) Sp.G.	62	172	70.0	23.7	11.4	80	101	160	63	270	300	IV
9) Ca.O.	57	164	74.0	27.6	14.8	180	104	166	72	92	240	HyperalphaLP
10) Na.P.	52	165	61.2	22.5	8.7	120	98	168	67	123	280	HyperalphaLP
11) D.C.A.	37	176	88.4	30.1	21.5	240	89	180	40	620	311	V
12) Mo.D.	46	171	69.3	25.7	9.4	100	93	217	56	155	263	HyperalphaLP
13) To.R.	55	170	112.0	38.7	46.8	240	108	163	78	453	267	IV HyperalphaLP
14) Ta.R.	48	167	63.1	22.7	9.5	180	98	158	82	50	212	Normal
15) Ci.S.	65	175	90.0	28.4	24.5	120	107	145	53	81	190	Normal
Mean ± SEM	51 ± 2	167 ± 1.3	71.1 ± 4.6	25.4 ± 1.4	15.9 ± 3.0	147 ± 16	95 ± 3	192 ± 10	73 ± 5	228 ± 53	283 ± 15	
Controls (15)	46 ± 2	167 ± 1.4	63.2 ± 3.8	22.5 ± 1.2	11.4 ± 2.6		92 ± 3	209 ± 9	48 ± 2	122 ± 16	234 ± 17	

[a] The LP phenotype was considered as hyperalphalipoproteinemia when HDL cholesterol values exceeded 70 mg/dl.

liquid ("added"). The ratio dpm-"recovery": dpm-"added" gives the percentage recovery in each experiment (15). The results were then corrected accordingly.

The chemical purity of the labeled triolein, checked by thin-layer chromatography on silica gel, was 98.7%.

Plasma post-heparin lipolytic activity (PHLA) was determined in 13 MSL patients (16). Blood samples were drawn before and 10 min after the injection of heparin, 60 U/kg body weight; 10 and 20 μl of pre- and post-heparin plasma were diluted in 1 ml of Krebs-Ringer-Tris buffer plus 1% bovine albumin and 10% human fasting serum plus 0.1 ml of substrate emulsion, as described above. The hepatic PHLA was determined by the same procedure, using Krebs-Ringer-Tris buffer with 1 M NaCl and without fasting human serum. Eight metabolically normal, slightly overweight subjects, matched for sex and age, volunteered for the studies on plasma post-heparin lipolytic activity.

Lipid and lipoprotein analysis

Cholesterol (17), triglycerides (18), phospholipids (19), apoprotein B, and A-I in serum and lipoprotein fractions were determined in 10 MSL patients and in 15 presumably normal subjects, matched for age, sex, and body weight. Apoproteins were determined by a modification of the rocket immunoelectrophoresis procedure of Laurell (20). The monospecific antibodies were prepared in our laboratory: anti-B in rabbit by injecting LP-B (d.1.035–1.045 g/ml) and anti A-I in sheep by injecting pure apoA-I obtained by column gel-chromatography on Sephadex G-150 (21) from ultracentrifugally separated (22) and delipidated (23) human HDL. Samples were delipidated by heating at 52°C for 3 hr.

Immunoelectrophoresis was carried out on 25 × 25 cm plates at 350 V for 4 hr for apoB and at 100 V for 30 hr for apoA-I. Wells were formed 3 cm from the lower edge and 100 mm distant from each other. Samples (10 μl) at optimal dilution for the antibody titer were placed in each well after heating at 52°C for 3 hr. The plates were dried and stained with Coomassie Brilliant Blue R-250. To determine apoA-I contents, a pool of sera stored at −20°C was used as working standard. ApoA-I content of working standard was calibrated against purified HDL$_3$ as primary standard. HDL$_3$ was isolated by ultracentrifugation at d 1.125–1.210 g/ml. Purity was tested by immunodiffusion, immunoelectrophoresis, and polyacrylamide gel electrophoresis. Protein content was determined by the method of Lowry et al. (24) and corrected by a factor of 0.60, corresponding to the mean concentration of apoA-I in the protein mass of HDL$_3$. Because of the uncertainty of this factor and the different chromogenicity of lipopro-
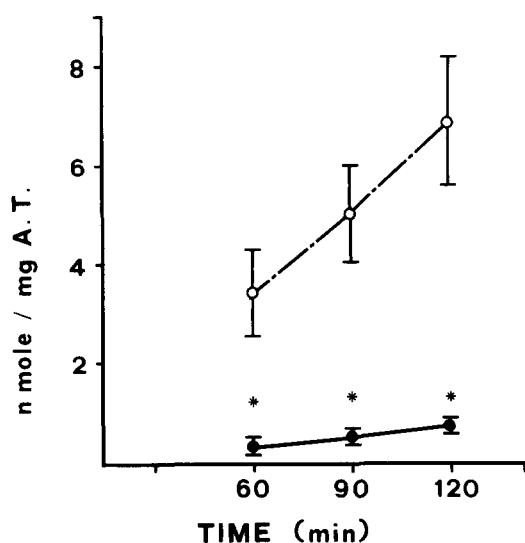
Fig. 2. Time course of the activity of LPL eluted from lipomatous (O –·– O) and normal (● —— ●) adipose tissue (vertical bars represent SEM; *, $P < 0.01$).

teins and apoproteins referred to albumin, the results were expressed in units/dl, where 1 unit of protein corresponded approximately to 1 mg. The apoB value of the pool was predetermined employing as primary standard the 1.035–1.045 g/ml fraction isolated by preparative ultracentrifugation.

Lipoprotein electrophoresis was carried out according to Seidel, Wieland, and Ruppert (25). Typing of the patients was performed according to W.H.O. criteria (26). Lipoprotein fractionation was carried out according to the N.I.H. method by preparative ultracentrifugation and selective polyanion precipitation (27). Cholesterol (17), triglyceride (18), and phospholipids (19) were determined on the different fractions obtained.

Polyacrylamide gel electrophoresis was performed on VLDL fractions for qualitative and semi-quantitative evaluation of apolipoproteins (28).

HDL$_2$ and HDL$_3$ subfractions were isolated from fasting plasma (EDTA 0.14%) by double polyanion precipitation according to Gidez et al. (29).

VLDL and LDL were precipitated from plasma with heparin, 1.26 mg/dl, and MnCl$_2$, 0.092 M. From the supernatant (HDL fraction), HDL$_2$ was separated from

HDL$_3$ by precipitation with dextran sulfate (15,000 molecular weight; Sochibo, Boulogne, France), 1.21% in 0.85% NaCl. Cholesterol in plasma and in HDL and HDL$_3$ fractions was determined according to Abell et al. (30). The HDL$_2$ cholesterol was calculated as the difference between HDL and HDL$_3$ cholesterol values.

Fat cell sizing

Fat cell sizing was performed on fresh tissue microsamples from needle biopsy (31). The triglyceride content of normal and lipomatous adipose tissue was determined gravimetrically after extraction with Dole's mixture (32). The fat cell number per mg of tissue was calculated from the ratio between the triglyceride content per mg of tissue and the mean triglyceride content per fat cell.

Data on intracellular FFA concentration, determined in adipose tissue of four MSL patients and in six controls, both in basal conditions and after drug-stimulated lipolysis, are unreported results of experiments of lipolysis previously published (for details see ref. 8).

Adipose tissue biopsies from uninvolved subcutaneous fat in most of MSL patients were unsuccessful because of the extremely reduced fat layer.

Statistical analysis

Statistical analysis was carried out using a nonparametric test for the comparison of the means and the multiple regression analysis for the evaluation of the influence of several variables on the level of HDL, HDL$_2$, and HDL$_3$.

RESULTS

Anthropometric data and routine metabolic parameters of MSL patients (Table 1)

None of the patients had abnormal fasting blood glucose levels. Four patients had type IV and two had type V hyperlipoproteinemia. The mean cholesterol values in MSL patients were normal. Plasma phospholipid levels were significantly higher than in controls. Mean plasma triglycerides were also higher in MSL, although the differences were statistically not significant because of the variance of the values in MSL group.

TABLE 2. Adipose tissue LPL activity and plasma PHLA in Multiple Symmetric Lipomatosis and in normal subjects (mean ± SEM)

	LPL Activity in Adipose Tissue (nmol/g per min)	Plasma PHLA ($\mu mol \cdot ml^{-1} \cdot hr^{-1}$)		
		Total	Hepatic	Extrahepatic
MSL	(10)[a] 56.7 ± 15.3**	(13) 25.4 ± 1.6	11.9 ± 1.4	13.8 ± 1.3*
Controls	(10) 6.2 ± 0.8	(8) 24.1 ± 2.2	15.2 ± 2.1	8.9 ± 1.3

* $P < 0.05$; ** $P < 0.01$.
[a] Number of experiments in parentheses.

TABLE 3. Composition of serum lipoproteins in MSL patients and in controls[a]

	Age	ApoB	ApoA-I	VLDL			LDL			HDL			HDL₂ CH	HDL₃ CH
				CH	TG	PL	CH	TG	PL	CH	TG	PL		
	yr	mg/dl	AU/dl											
MSL (15)	51 ± 2	95 ± 5	209 ± 11**	21 ± 6	122 ± 38	21 ± 9	90 ± 8**	57 ± 14	94 ± 7	71 ± 5**	27 ± 3	149 ± 10**	31 ± 4**	40 ± 3*
Controls (15)	46 ± 2	90 ± 2	160 ± 3	11 ± 2	51 ± 4	11 ± 3	148 ± 8	33 ± 6	97 ± 7	48 ± 2	23 ± 2	92 ± 7	14 ± 1	33 ± 1

*, $P < 0.05$; **, $P < 0.001$.
[a] Results are expressed as mg/dl ± SEM. AU, arbitrary units; CH, cholesterol; TG, triglyceride; PL, phospholipid.

LPL activity in adipose tissue

The time course of the activity of LPL eluted from lipomatous and normal adipose tissue is represented in **Fig. 2.** As reported in **Table 2,** average LPL activity, expressed in nmol/g per min in lipomatous tissue was about ten times higher than in normal tissue (56.7 ± 15.3 nmol/g per min vs. 6.2 ± 0.8 nmol/g per min; $P < 0.05$; ranges: 10.9–180.4 nmol/g per min vs. 1.4–9.5 nmol/g per min).

Plasma PHLA (Table 2)

Total and hepatic PHLA were statistically not different in MSL patients and in controls, while extrahepatic PHLA was significantly higher ($P < 0.05$) in MSL patients than in controls. No correlations were found between LPL activity in adipose tissue and plasma total or extrahepatic PHLA activities.

Lipoprotein composition (Table 3)

Apoprotein B levels were normal, while apoA-I levels were significantly higher in MSL patients than in controls. VLDL values were increased in MSL patients; cholesterol and triglyceride content in VLDL was almost doubled as compared to controls, due to the six hyperlipidemic type IV and V subjects in the MSL group.

LDL had an abnormal composition in MSL patients. Mean LDL cholesterol in MSL was significantly lower than in controls ($P < 0.01$). In this fraction the CH/PL ratio in MSL patients (0.97 ± 0.10) was significantly lower than in normal subjects (1.52 ± 0.10; $P < 0.001$).

HDL values, according to the N.I.H. method (27), were significantly higher in MSL patients than in controls ($P < 0.01$) (Table 3). In this fraction, the CH/PL ratio was almost the same as in normal subjects and in familial hyperalphalipoproteinemia (33). HDL₂ cholesterol values were twice those of controls; the difference was statistically significant ($P < 0.001$). HDL₃ cholesterol was also significantly increased in MSL patients ($P < 0.05$), but to a lower extent than HDL₂ cholesterol; thus, HDL₂/HDL₃ cholesterol ratio in MSL patients was significantly higher than in controls (0.87 ± 0.13 vs. 0.44 ± 0.04; $P < 0.01$).

Polyacrylamide gel electrophoresis did not demonstrate abnormalities of C-II and C-III apolipoproteins.

A significant correlation was found in MSL patients between the LPL activity in adipose tissue and the plasma values of HDL₂ cholesterol (**Fig. 3**), as well as between VLDL triglycerides and HDL₂ cholesterol ($r = -0.54$, $P < 0.05$). No statistically significant correlations were demonstrated between body fat mass, fat cell size, plasma cholesterol, triglyceride, or phospholipid values and adipose tissue LPL or plasma PHLA
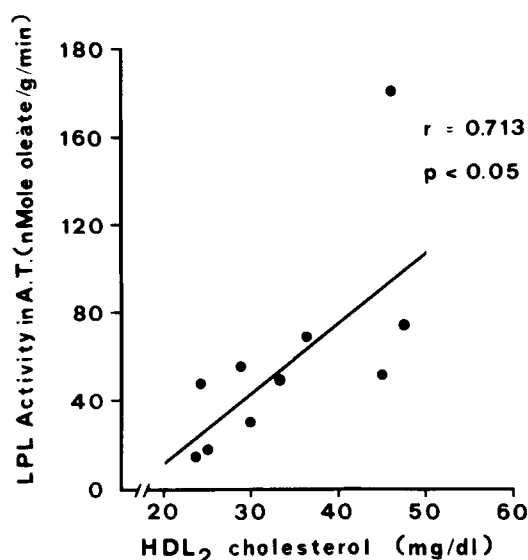
Fig. 3. Correlation between LPL activity in adipose tissue and plasma HDL$_2$-cholesterol levels in MSL.

activities. Multiple regression analysis revealed that neither age nor alcohol intake contributed significantly to the increments of HDL and HDL$_2$ levels.

Fat cell sizing (Table 4)

The mean weight of fat cells from lipomatous tissue was lower, but not significantly different, than fat cells of the normal fat tissue. No differences in mean triglyceride content per mg of adipose tissue and in mean fat cell number per g of tissue were found in adipose tissue from MSL patients and from controls. Therefore, the results of adipose tissue LPL activity have been expressed only per unit of weight of fresh tissue, not per cell.

FFA content in lipomatous tissue in basal conditions and after theophylline or dibutyril-cyclic AMP addition to the medium was not different in comparison to normal tissue. After isoprenaline addition, no intracellular accumulation of FFA in lipomatous tissue was observed (Table 4).

DISCUSSION

The abnormal accumulation of fat in lipomatous tissue of MSL patients, which gives rise to fatty tumors, would suggest alterations in the lipid deposition in some fat cell precursors.

A specific defect in catecholamine-induced lipid mobilization was demonstrated in fat cells from lipomatous tissue. The observations of a normal hormone-sensitive lipase (HSL) activation after theophylline and dibutyril-cyclic AMP, of normal intracellular levels of ATP (8) and of a normal production of 3,5-cyclic AMP after adrenergic stimulation (9) suggest multiple or differently located defects in the lipolytic pathway. Furthermore, the finding of a normal lipolytic response of lipomatous tissue to cholera toxin (34) seems to exclude defects in the adenylate-cyclase system. Possible alterations in β-receptor activity have been postulated but not confirmed (10, 12). Moreover, sporadic observations of increased LPL activity in experiments on lipomatous tissue from one patient have been reported (11). A dramatic increase in LPL activity in lipomatous tissue was demonstrated in the present work on a number of MSL patients large enough to give definitive information. Thus, in addition to the previously well documented defect in adrenergic-stimulated lipid mobilization, a further possible mechanism of triglyceride accumulation in adipose tissue, represented by an increased LPL-dependent FFA incorporation, can be postulated. It seems rather unusual that two different errors in metabolic regulation could co-exist in the same cells, both leading to the same final effect. More probably, a reciprocal modulation between HSL and LPL activities would exist.

Alterations in LPL activity in adipose tissue induced by fasting and feeding correlate inversely with the concomitant effects on HSL activity (35–38), while an inverse regulation of HSL and LPL activities was demonstrated in rat adipocytes during HSL activation (39). A LPL-mediated intracellular FFA accumulation in lipomatous fat cells responsible for HSL suppression has

TABLE 4. Characteristics of adipose tissue in MSL patients and in normal controls

| | FCW (µg TG) | TG (g)[a] | FCN (10⁶)[a] | Intracellular FFA (µEq/g of fresh tissue/150 min) | | | |
				Basal (No drugs)	Isoprenaline 10⁻⁵ M	cAMPdb 10⁻³ M	Theophylline 3 × 10⁻³ M
MSL	0.58 ± 0.07[b]	0.74 ± 0.02	1.32 ± 0.2	1.67 ± 0.42	1.48 ± 0.10	6.18 ± 0.14	9.00 ± 0.42
Controls	0.65 ± 0.08	0.79 ± 0.01	1.21 ± 0.1	2.21 ± 0.70	5.63 ± 0.38*	6.25 ± 0.20	7.93 ± 0.86

Fat cell weight (FCW), triglyceride (TG) content, and fat cell number (FCN) of adipose tissue in 13 MSL patients and in 8 controls, and intracellular levels of FFA in adipose tissue before and after drug-stimulated lipolysis in 4 MSL patients and in 6 controls.

*, $P < 0.001$.
[a] Per gram of adipose tissue.
[b] Mean ± SEM.

to be considered. Intracellular FFA concentration seems to be the modulator of lipolysis (40). FFA inhibits lipolysis in rat and human fat cells, probably by acting as feedback regulator of adenylate-cyclase and cyclic-3',5'-AMP accumulation (41, 42), or by increasing NADH with inhibitory effects on adenylate-cyclase activity (43). In our experiments, intracellular levels of FFA in lipomatous tissue were not different from those in control tissue both in basal condition and after dibutyril cyclic-AMP or theophylline-stimulated lipolysis, while no increase in intracellular FFA concentration was present after isoprenaline stimulation. From these observations a role of intracellular FFA accumulation in HSL suppression can be excluded.

Whatever the reciprocal regulation between HSL and LPL activities, MSL represents a naturally occurring model of a primarily increased LPL activity in adipose tissue. In normal subjects as well as in patients with familial hyperalphalipoproteinemia, a close correlation was demonstrated between LPL activity in adipose tissue and plasma HDL values (44, 45). The same relationship was found even in MSL patients, which confirms that LPL activity in adipose tissue is closely related to the circulating levels of HDL, especially HDL_2. Furthermore, the statistically significant inverse correlation between VLDL-triglycerides and HDL-cholesterol can help to explain the elevated levels of HDL and HDL_2 in MSL patients. In fact, it has been demonstrated that the HDL constituents (apoA-I, fatty acids, phospholipids) are derived from the triglyceride-rich lipoproteins during their catabolism (46, 47).

In the present study no correlation was found between the in vitro releasable adipose tissue LPL and the plasma LPL released in vivo after heparin injection. This would be explained by a dilution of the LPL of adipose tissue in the larger pool of heparin-delivered circulating lipases from several different tissues or by large variations of the total amount of lipomatous tissue from patient to patient or by a delayed heparin-stimulated LPL release from adipose tissue in vivo (48).

Increased levels of HDL in chronic alcoholics is well documented (49–52). The ethanol-dependent HDL increase in plasma is associated with an increase in LPL activity in adipose tissue (44). Nevertheless, the rather elevated alcohol intake in MSL patients can only partially explain the marked increments in HDL levels. The estimated alcohol consumption of 60–220 ml a day does not frankly exceed the customary intake for male adults of that region, age, and social class. In a population sample of the same sex, age, and provenance, the increase in serum apoA-I values in heavy drinkers did not exceed the 10% of the values for nondrinkers (53). Moreover, in MSL patients, no correlation was found between the daily alcohol intake and HDL or HDL_2

plasma levels; at multiple regression analysis, neither age nor ethanol intake contributed significantly to the increments of HDL or HDL_2 levels.

The high prevalence of type IV and V hyperalphalipoproteinemia can be explained by an alcohol-induced hepatic synthesis of VLDL. Nevertheless, in MSL patients, HDL, HDL_2, and apoA-I were constantly elevated, whatever the lipoprotein phenotype: normal, type IV, or type V. This supports the diagnosis of a primary type of hyperalphalipoproteinemia.

The significantly lower levels of total LDL and their abnormal composition in MSL patients have never been reported previously. Hypothetically, even these abnormalities in LDL composition might be due to the chronically increased activity of LPL derived from adipose tissue.

Several familial cases of MSL have been reported (5, 54–56) and a familial occurrence of the disease was also present in our series (Table 1: M.G., no. 2 and M.D., no. 3, brothers). Although no male-to-male transmission was observed, the disorder has been considered as probably autosomal dominant (57). Thus, an inherited enzymatic defect can be assumed responsible for a "triglyceride-storage disease" in adipose tissue. Metabolic studies in unaffected relatives of MSL patients could probably identify the primary regulatory defect responsible for the abnormal triglyceride accumulation in lipomatous tissue.∎

REFERENCES

1. Strange, D. A., and W. J. Fessel. 1968. Benign symmetric lipomatosis. *J. Am. Med. Assoc.* **204:** 339–340.
2. Heully, F., J. Lauren, M. A. Bigard, J. P. Gonand, and J. Gillot. 1969. Anomalies du métabolisme lipidique et glucidique au cours d'une lipomatose de Launois-Bensaude. *Presse Med.* **77:** 2001–2002.
3. Colwell, J. A., and S. R. Cruz. 1972. Effects of resection of adipose tissue on the diabetes and hyperinsulinism of benign symmetrical lipomatosis. *Diabetes.* **21:** 13–15.
4. Springer, H. A., and J. S. Whitehouse. 1972. Launois-Bensaude adenolipomatosis. *Plast. Reconstr. Surg.* **50:** 291–294.
5. Green, M. L., C. J. Glueck, M. Y. Fuyimoto, and J. E. Seegmiller. 1970. Benign symmetric lipomatosis (Launois-Bensaude adenolipomatosis) with gout and hyperlipoproteinemia. *Am. J. Med.* **48:** 239–246.
6. Muller, M. N., and O. Frank. 1974. Lipid and purine metabolism in benign symmetric lipomatosis. *Adv. Exp. Med. Biol.* **41:** 509–516.
7. Grivaux, M., M. Gentilini, J. Soulie, and P. Badoual. 1969. Adenolipomatose symmetrique de Launois-Bensaude et troubles du métabolisme lipidique. *Sem. Hop. Paris.* **45:** 1958–1966.
8. Enzi, G., E. M. Inelmen, A. Baritussio, P. Dorigo, M.

Prosdocimi, and F. Mazzoleni. 1977. Multiple symmetric lipomatosis. A defect in adrenergic-stimulated lipolysis. *J. Clin. Invest.* **60:** 1221–1229.

9. Desai, K. S., B. Zinman, and G. Steiner. 1979. Multiple symmetric lipomatosis (Launois-Bensaude syndrome). Adipose tissue insensitivity to cyclic AMP. *J. Clin. Endocrinol. Metab.* **49:** 307–310.

10. Pecquery, R., L. Malagrida, and Y. Giudicelli. 1980. Adipocyte adenylate cyclase and alpha- and beta-adrenergic receptors in one case of multiple symmetric lipomatosis. *Biomedicine.* **33:** 64–66.

11. Giudicelli, Y., R. Pecquery, B. Agli, C. Jamin, and J. Quevanvilliers. 1976. Lipoprotein lipase and hormone-sensitive lipase activities in human subcutaneous lipomas: comparison with normal subcutaneous adipose tissue. *Clin. Sci. Molec. Med.* **50:** 315–318.

12. Smith, U. 1981. Adipose tissue metabolism in some pathophysiolocal states in man. *In* Recent Advances in Obesity Research. III P. Björntorp, M. Cairella, and A. N. Howard, editors. John Libbey, London. 357–360.

13. Edwards, K. D., and H. M. White. 1962. The simple measurement of obesity. *Clin. Sci.* **22:** 347–352.

14. Pykälisto, O. J., P. H. Smith, and J. D. Brunzell. 1975. Determinants of human adipose tissue lipoprotein lipase. *J. Clin. Invest.* **56:** 1108–1117.

15. Belfrage, P., and M. Vaughan. 1969. Simple liquid-liquid partition system for isolation of labeled oleic acid from mixtures with glycerides. *J. Lipid Res.* **10:** 341–344.

16. Ehnholm, C., H. Greten, and W. V. Brown. 1974. A Comparative study of postheparin lipolytic activity and a purified human plasma triacylglycerol lipase. *Biochim. Biophys. Acta.* **360:** 68–77.

17. Röschlau, P., E. Berndt, and W. Gruber. 1974. Enzymatische Bestimmung des Gesamt-Cholesterin in Serum. *Z. Klin. Chem. Klin. Biochem.* **12:** 226.

18. Wahlefeld, A. W. 1978. Triglyceride determination after enzymatic hydrolysis. *In* Methoden der Enzymatische Analyse. H. V. Bergmeyer, editor. Verlag Chemie, Weinheim. 1878.

19. Zilversmit, D. B., and A. K. Davis. 1950. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. *J. Lab. Clin. Med.* **35:** 155–163.

20. Laurell, C. B. 1966. Quantitative estimation of protein by electrophoresis in agarose gel containing antibodies. *Anal. Biochem.* **15:** 45–52.

21. Jackson, R. L., and A. M. Gotto. 1972. A study of the cystine-containing apolipoprotein of human plasma high density lipoproteins; characterization of cyanogen bromide and tryptic fragments. *Biochim. Biophys. Acta.* **285:** 26–47.

22. Havel, R. J., H. A. Eder, and J. H. Bragdon. 1955. The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.* **34:** 1345–1353.

23. Scanu, A. M., and C. Edelstein. 1971. Solubility in aqueous solutions of ethanol of the small molecular weight peptides of the serum very low density and high density lipoproteins: relevance to recovery problem during delipidation of serum lipoproteins. *Anal. Biochem.* **44:** 576–588.

24. Lowry, N. O., N. J. Roseburg, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193:** 265–275.

25. Seidel, D., H. Wieland, and C. Ruppert. 1973. Improved techniques for assessment of plasma lipoprotein patterns. I. Precipitations in gels after electrophoresis with polyanionic compounds. *Clin. Chem.* **19:** 737–743.

26. Beaumont, J. L., L. A. Carlson, G. R. Cooper, Z. Fejfar, and D. G. Fredrickson. 1970. Classification of hyperlipidemias and hyperlipoproteinemia. *Bull. WHO.* **43:** 891–899.

27. Manual of Laboratory Operations. 1974. Lipid Research Clinics Program. N.I.H. Lipid and Lipoprotein Analysis **1:** 56–58.

28. Kane, J. P. 1973. A rapid electrophoretic technique for identification of subunit species of apoproteins in serum lipoproteins. *Anal. Biochem.* **53:** 350–364.

29. Gidez, L. I., G. J. Miller, M. Burstein, and H. A. Eder. 1979. Analyses of plasma high density lipoprotein subclasses by a precipitation procedure: correlation with preparative and analytical ultracentrifugation. *In* Report of the High Density Lipoprotein Methodology. K. Lippel, editor. Workshop U.S. Department of Health, Education and Welfare. N.I.H. Publication, no. 79-1661. 328–340.

30. Abell, L. L., B. B. Levy, B. B. Brodie, and F. E. Kendall. 1952. A simplified method for the estimation of total cholesterol in serum and demonstration of its specificity. *J. Biol. Chem.* **195:** 357–366.

31. Sjöström, L., P. Björntorp, and J. Vrana. 1971. Microscopic fat cell size measurements on frozen-cut adipose tissue in comparison with automatic determinations of osmium-fixed fat cells. *J. Lipid Res.* **12:** 521–530.

32. Dole, V. P. 1956. A relation between nonesterified fatty acids in plasma and the metabolism of glucose. *J. Clin. Invest.* **35:** 150–154.

33. Mendoza, S., R. F. Lutmer, C. J. Glueck, C. Y. Chen, P. M. Steiner, R. W. Fallat, and M. L. Kashyap. 1976. Composition of HDL_2 and HDL_3 in familial hyperalphalipoproteinemia. *Atherosclerosis.* **25:** 131–137.

34. Dorigo, P., M. Prosdocimi, F. Carpenedo, L. Caparrota, F. Tessari, and G. Enzi. 1980. Multiple symmetric lipomatosis. A defect in adrenergic-stimulated lipolysis II. *Pharmacol. Res. Commun.* **12:** 625–638.

35. Hollenberg C. H. 1959. Effect of nutrition on activity and release of lipase from rat adipose tissue. *Am. J. Physiol.* **197:** 667–670.

36. Wing, D. R., and D. S. Robinson. 1968. Clearing-factor lipase in adipose tissue. A possible role of adenosine-3', 5'-cyclic-monophosphate in the regulation of its activity. *Biochem. J.* **109:** 841–849.

37. Robinson D. S., and D. R. Wing. 1970. Regulation of adipose tissue clearing factor lipase activity. *Horm. Metab. Res.* **2, Suppl. 2:** 41–46.

38. Robinson D. S., and D. R. Wing. 1971. Studies on tissue clearing lipase related to its role in the removal of lipoprotein triglyceride from the plasma. *Biochem. Soc. Symp.* **33:** 123–135.

39. Patten R. L. 1970. The reciprocal regulation of lipoprotein lipase activity and hormone-sensitive lipase activity in rat adipocytes. *J. Biol. Chem.* **245:** 5577–5584.

40. Rodbell, M. 1965. Modulation of lipolysis in adipose tissue by fatty acid concentration in fat cells. *Ann. NY Acad. Sci.* **131:** 302–314.

41. Fain, J. N., and R. E. Shepherd. 1975. Free fatty acids as feedback regulators of adenylate cyclase and cyclic-3':5'-AMP accumulation in rat fat cells. *J. Biol. Chem.* **250:** 6586–6592.

42. Burns, T. W., P. E. Langley, and G. A. Robinson. 1975.