Insulin deficiency and reduced expression of lipogenic enzymes in cardiomyopathic hamster

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Abstract Evidence is given that the heart of the cardiomyopathic UM-X7.1 hamster has a lipid composition different from that of the same tissue isolated from animals of the Syrian hamster parent strain. Also, noncardiac tissues from cardiomyopathic and healthy hamsters exhibit significant compositional differences. On the basis of these preliminary observations, a comparative study of the hepatic biosynthesis of lipids in cardiomyopathic and healthy Syrian hamsters was undertaken. The results obtained indicate that the cardiomyopathic hamster is characterized by a generalized disturbance of lipid metabolism. In particular, the fatty acid synthase and stearoyl-CoA desaturase activities were significantly lower in the liver of UM-X7.1 hamsters than in age-matched healthy controls fed the same diet. Northern blot analysis of the mRNAs encoding the two enzymatic proteins and the "lipogenic" S14 nuclear protein indicated that the transcription of the respective genes was impaired in UM-X7.1. Short-term dietary manipulations modulated the expression of the above-mentioned genes both in cardiomyopathic and healthy animals. However, dietary carbohydrates were less effective in inducing the expression of lipogenic enzymes in UM-X7.1 liver than healthy controls. The main determinant of the metabolic defect pointed out in the present work appears to be represented by the low insulin level detectable in the plasma of the cardiomyopathic hamster.—Vecchini, A., L. Binaglia, M. Bibeau, M. Minieri, F. Carotenuto, and P. Di Nardo. **Insulin deficiency and reduced expression of lipogenic enzymes in cardiomyopathic hamster.** ***J. Lipid Res.*** **2001.** 42: **96–105.**

Supplementary key words fatty acid synthase • stearoyl-CoA desaturase • gene expression • diet

During the last 30 years the cardiomyopathic hamster (CMPH) has represented a widely used animal model for studying the physiological and biochemical determinants of hypertrophic cardiomyopathy (1). Nigro et al. (2) have demonstrated that, because of a mutation of the gene encoding δ-sarcoglycan (δ-SG), the content of δ-SG mRNA is low both in the skeletal muscle and in the heart of CMPH and that the encoded protein is not detectable in the same tissues by Western blot analysis. Such a genetic defect is considered to be the primary cause of the hamster cardiomyopathy.

In the present study, we give evidence that the CMPH is characterized also by a generalized disturbance of lipid metabolism and that such a metabolic defect could originate from its low level of plasmatic insulin.

Indeed, it is well known that, in addition to its function in modulating the intracellular metabolism, the major role of insulin is represented by its involvement in the regulation of the expression of a wide series of genes (3, 4). Among the genes whose transcription is positively regulated by insulin are those encoding acetyl-CoA carboxylase, fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD1), *sn*-glycerol-3-phosphate acyltransferase, and the nuclear lipogenic protein S14. In addition to insulin, dietary carbohydrates also positively modulate the transcription of the same lipogenic genes whereas polyenoic fatty acids play the opposite role (5–7). As a consequence of this many-sided regulation, and because of the close interaction existing between dietary carbohydrates and circulating insulin level, it is often difficult to discriminate between hormonal and nutritional effects when performing animal studies. Therefore, the insulin deficiency of CMPH could represent a valuable model for separately analyzing in vivo the modulatory role of the two factors in the expression of lipogenic genes.

Evidence is given in the present work that dietary manipulations are able to correct the metabolic differences existing between cardiomyopathic and healthy hamsters.

Abbreviations: CMPH, cardiomyopathic hamster; FAS, fatty acid synthase; HPLC, high performance liquid chromatography; PBS, phosphate-buffered saline; PtdCho, phosphatidylcholine; PtdEtn, phosphatidylethanolamine; PPARα, peroxisome proliferator-activated receptor α; PUFA, polyunsaturated fatty acids; RP-HPLC, reversed phase HPLC, SCD1, stearoyl-CoA desaturase.

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MATERIALS AND METHODS

[2-^{14}C]acetate (specific activity, 2 Ci/mmol) was purchased from New England Nuclear (Boston, MA). [2-^{3}H]glycerol (specific activity, 1 Ci/mmol) was obtained from Amersham (Arlington Heights, IL). The cDNA probe for SCD1 was kindly supplied by J. Ntambi (Department of Biochemistry, University of Wisconsin, Madison, WI) whereas cDNA probes for FAS and S14 were obtained from D. B. Jump (Department of Biochemistry, Michigan State University, East Lansing, MI). All other chemicals were purchased from Sigma-Aldrich (Milan, Italy).

Animal treatments

Syrian cardiomyopathic hamsters (UM-X7.1 strain) (8) and healthy golden Syrian hamsters employed in this study as experimental animals were bred in our animal facility under the same conditions. When not otherwise stated, animals were fed with a standard pellet chow (Rieper, Bolzano, Italy) and water was supplied ad libitum. The daily food intake of cardiomyopathic hamsters (8.4 ± 0.5 g per 100 g body weight) was not significantly different from that of controls (8.5 ± 0.4 g per 100 g body weight). The animals were killed by decapitation when aged 20, 90, or 150 days. The heart and other tissues examined were rapidly excised, frozen in liquid nitrogen, and kept at −80°C before being analyzed.

Glucose plasma level was determined according to standard techniques. Serum insulin was assayed with a rat insulin radioimmunoassay kit (Linco Research, St. Charles, MO) according to the manufacturer directions. The antibody against rat insulin displayed a 100% cross-reactivity for hamster insulin.

Lipid analyses

Tissues were homogenized in water at 4°C, using an Ultra-Turrax T25 homogenizer (6 × 15 s, setting 5; Janke & Kunkel, Staufen, Germany). Proteins were quantitated in aliquots of the homogenates according to Lowry et al. (9). Lipids were extracted from aliquots of the same homogenates according to Folch, Lees, and Sloane-Stanley (10). The lipid extracts were evaporated under vacuum and the lipid residues were dissolved in benzene and stored at −20°C under nitrogen.

Phospholipid classes were isolated by two-dimensional thin-layer chromatography on silica gel G plates according to Horrocks (11). Neutral lipids were isolated by thin-layer chromatography on silica gel G plates, using diethylether–hexane–acetic acid 50:50:1 (v/v/v) as developing mixture.

Phospholipids were quantitated as inorganic phosphate, after mineralization, using the method described by Baykov, Evtushenko, and Avaeva (12). Cholesterol was quantitated according to Mascini, Tomassetti, and Iannello (13).

The analysis of phosphatidylcholine (PtdCho) and phosphatidylethanolamine (PtdEtn) molecular species was performed according to a previously published procedure (14). In particular, diacylglycerols were prepared by phospholipase C digestion of the phospholipid and purified by high performance liquid chromatography (HPLC) on silicic acid. The individual molecular species of diacylglycerol were separated by reversed phase (RP)-HPLC.

The HPLC separation of labeled fatty acid methyl esters was carried out according to Aveldano, van Rollins, and Horrocks (15) on a 2.1 mm × 15 cm octadecyl-silica column (Supelcosil LC-18; Supelco, Bellefonte, PA), using a model 2248 Pharmacia-LKB pump (Amersham Pharmacia Biotech, Uppsala, Sweden) equipped with a model DDL-21 evaporative light scattering detector (Cunow, Cergy-St-Christophe, France) and a Packard (Meriden, CT) model Flow-One A-100 flowthrough radioactivity detector. The temperature of the column was kept at 5°C using a Violet (Rome, Italy) model T-55 temperature control module. A Violet model GR-30 programmer equipped with a 100T3 solenoid-operated three way valve (Bio-Chem Valve, Chichester, UK) was used to split the eluates from the column between the two detectors. The signals obtained from the two detectors were recorded in two parallel channels of a model C-RSA Chromatopac (Shimadzu, Kyoto, Japan) C-R4A integrating unit.

Gas chromatography of fatty acid methyl esters was performed with a Carlo Erba Instruments (Milan, Italy) model HRGC 5300 gas chromatograph equipped with an SP-2330 capillary column (30 m × 0.25 mm; Supelco) and flame ionization detector.

Glycerolipid synthesis from [2-^{3}H]glycerol

Twenty-day-old CMPH (n = 4, 33.4 ± 6.8 g body weight) were injected intraperitoneally with 100 μCi of [2-^{3}H]glycerol in 0.5 ml of phosphate-buffered saline (PBS). The hamsters were killed at times ranging from 10 min to 2 h after the injection. Age-matched healthy Syrian hamsters (n = 4, 46.9 ± 7.3 g body weight) received the same isotope injection and were killed after the same time intervals. The livers were excised and washed with saline, and lipids were extracted according to Folch, Lees, and Sloane-Stanley (10). The labeling of single lipid classes, separated as described above, was assessed by liquid scintillation counting (16).

Fatty acid synthesis from [2-^{14}C]acetate

Twenty-day-old cardiomyopathic (n = 4, 32.4 ± 5.9 g body weight) and control (n = 4, 48.0 ± 4.6 g body weight) hamsters were injected intraperitoneally with 30 μCi of [2-^{14}C]acetate in 0.5 ml of PBS and killed at times ranging from 10 min to 2 h after injection. Liver lipids were extracted and isolated as described above. The labeling of total lipids and of the lipid classes isolated by thin-layer chromatography was measured by liquid scintillation counting.

Aliquots (20%) of each total lipid extract were subjected to transmethylation as described above. The labeled methyl esters obtained were isolated chromatographically and the incorporation of the labeled precursor in the whole fatty acids was determined by liquid scintillation counting.

Individual fatty acid methyl esters were separated by HPLC on a 2.1 mm × 15 cm octadecyl-silica column (Supelcosil LC-18; Supelco), using an acetonitrile–water 96:4 (v/v) mixture as mobile phase (flow rate, 0.3 ml/min). The fatty acids contained in the labeled peaks eluted from the HPLC column were analyzed by gas chromatography. The pure fractions eluted from the HPLC column were evaporated and the radioactivity was determined by liquid scintillation counting. Labeled palmitoleic and eicosatrienoic acid methyl esters, which coeluted from the column, were separated by thin-layer chromatography on silica gel G impregnated with 10% silver nitrate, according to Christie (17). The silica scrapes containing the individual fatty acid methyl esters were extracted and counted for radioactivity as described previously (14).

Northern blot analyses

Twelve-microgram samples of total RNA, extracted from the livers of healthy hamsters and CMPH (18), were electrophoretically separated on 1.2% agarose under denaturing conditions and transferred to nylon membranes. Membranes were hybridized in a Quickhyb solution (Stratagene, La Jolla, CA) with different ^{32}P-labeled cDNA probes at 68°C. The filters were washed at room temperature with 2× saline sodium citrate (SSC) plus 0.1% sodium dodecyl sulfate (SDS) and then with 0.1% SSC + 0.1% SDS at 60°C. After hybridization, gels were dried and subjected to autoradiography. HL11.3 housekeeping cDNA was used as a probe to check for the integrity and the amount of loaded RNAs. The following probes were used: SCD1 250 base

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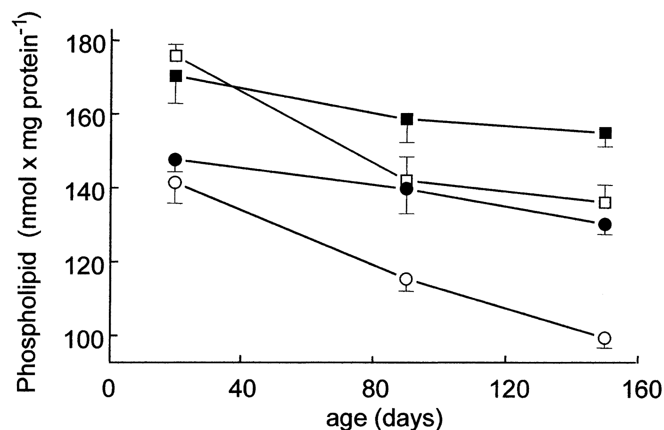

Fig. 1. Age-related changes in the phospholipid-to-protein ratio in left (circles) and right (squares) cardiac ventricles of cardiomyopathic (open symbols) and healthy (filled symbols) Syrian hamsters.

parts (bp) cloned in pGEM, FAS 800 bp cloned in pBR322, and S14 498 bp cloned in pGEM-1.

Effect of high carbohydrate and vegetal diets on de novo fatty acid synthesis in liver

After 12 h of fasting, 3-month-old cardiomyopathic (n = 3, 85.3 ± 7.1 g body weight) and control (n = 3, 150.9 ± 16.9 g body weight) hamsters were fed for 48 h with a fat-free high carbohydrate diet (fructose/bran, 8:2, by weight). Two other groups of hamsters were fed for 48 h with a low fat vegetal diet (fresh apples and carrots). Two additional groups were fed a conventional pellet diet. The animals were injected intraperitoneally with 100 μCi of [2-^{14}C]acetate in saline and killed 15 min after isotope administration.

The label incorporation into liver lipids and the content of the mRNA encoding FAS, SCD, and protein S14 were determined as described above.

RESULTS

Heart lipid composition

The right ventricles of 20-day-old cardiomyopathic and control hamsters exhibited a significantly higher phospholipid-to-protein ratio than left ventricles. After weaning, the phospholipid-to-protein ratio progressively decreased in both ventricles of cardiomyopathic and control hamsters. Such a decrease was higher in CMPH than in control animals (**Fig. 1**). PtdCho and PtdEtn were the phospholipid classes mainly involved in the aforementioned age-related changes (**Table 1**). In fact, the cardiac content of PtdCho per milligram of protein decreased by about 12% in the healthy animals during the age interval of 20 to 150 days, whereas it decreased as much as 31% and 35%, respectively, in the right and left ventricles of the cardiomyopathic animals. During the same age interval, the PtdEtn content per milligram of protein decreased by about 7% in the hearts of healthy animals, whereas it decreased by as much as 24–27% in the hearts of CMPH. The cholesterol content of the whole heart was not significantly different in 20-day-old cardiomyopathic and control hamsters. On the other hand, the heart of 3-month-old CMPH exhibited a higher cholesterol content than healthy controls, this difference not being evident at the fifth month of age (Table 1). The molecular species composition of heart PtdEtn was not dissimilar in 90-day-old cardiomyopathic and healthy hamsters, except for 18:0/20:4 species, the percent content of which was about 1.5 times higher in CMPH than in healthy controls. More relevant differences between the two hamster populations were observed when the mass distribution among the species of liver PtdCho was considered. Indeed, the percent content of both 1-stearoyl-2-linoleoyl-*sn*-glycerol-3-phosphocholine and 1-stearoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine was more than two times higher in

TABLE 1. Phospholipid and cholesterol content of the heart from cardiomyopathic UM-X7.1 and healthy Syrian hamsters at different ages

	Age (days)					
	20		90		150	
	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1
Right ventricle						
PtdEtn	57.72 ± 1.99	57.51 ± 0.82	54.38 ± 2.19	45.58 ± 1.16[a]	53.28 ± 0.96	41.83 ± 2.21[a]
PtdCho	64.02 ± 2.67	65.99 ± 0.31	60.04 ± 1.55	50.03 ± 3.35[a]	56.19 ± 1.36	45.74 ± 2.40[a]
PtdSer	7.59 ± 0.49	8.41 ± 1.46	5.83 ± 0.52	8.23 ± 0.10[a]	6.21 ± 0.62	8.78 ± 0.69[a]
PtdIns	17.49 ± 0.99	15.81 ± 0.25	17.31 ± 0.69	15.90 ± 0.49	15.04 ± 0.51	15.20 ± 0.09
SM	9.83 ± 0.37	11.54 ± 0.76	6.92 ± 1.73	11.12 ± 0.55[a]	8.26 ± 0.11	11.04 ± 0.18[a]
CL	13.58 ± 0.43	15.97 ± 0.92	14.2 ± 0.77	10.72 ± 0.71[a]	16.09 ± 0.17	13.52 ± 0.16[a]
Left ventricle						
PtdEtn	48.78 ± 3.09	46.90 ± 3.70	50.87 ± 4.94	38.08 ± 1.45[a]	45.37 ± 0.94	35.41 ± 0.81[a]
PtdCho	58.13 ± 2.81	54.08 ± 2.49	51.28 ± 3.15	39.57 ± 0.66[a]	51.16 ± 2.72	35.08 ± 1.32[a]
PtdSer	6.25 ± 0.86	6.31 ± 0.51	5.91 ± 1.01	6.46 ± 0.22[b]	4.45 ± 0.53	5.38 ± 0.25
PtdIns	13.62 ± 1.09	13.95 ± 14.97	14.97 ± 1.27	12.66 ± 1.36	11.54 ± 1.04	10.39 ± 0.47
SM	9.52 ± 1.26	8.35 ± 0.45	5.95 ± 0.71	6.46 ± 0.52	4.60 ± 0.24	6.11 ± 0.51[b]
CL	11.36 ± 1.40	11.89 ± 0.94	11.79 ± 1.14	12.24 ± 0.85	13.20 ± 0.57	7.16 ± 0.81[a]
Cholesterol (whole heart)	46.5 ± 6.4	44.2 ± 2.9	45.1 ± 5.4	54.6 ± 3.2[b]	60.4 ± 6.2	53.7 ± 4.9

Data, obtained from the analysis of three separate samples, are expressed in nmol·mg protein^{-1}. Ptd Ser, phosphatidylserine; PtdIns, phosphatidylinositol; SM, sphingomyelin; CL, cardiolipin. Significantly different from age-matched controls ([a] $P < 0.005$; [b] $P < 0.01$).

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TABLE 2. Molar percent composition of individual fatty acids obtained by trans-esterification of the whole lipids from the heart ventricles of cardiomyopathic UM-X7.1 and healthy Syrian hamsters.

	Age (days)					
	20		90		150	
	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1
Left ventricle						
16:0	21.78 ± 1.77	17.23 ± 1.20	17.37 ± 1.62	17.11 ± 1.34	16.66 ± 0.96	16.70 ± 1.60
16:1	0.92 ± 0.33	0.89 ± 0.42	0.70 ± 0.10	0.71 ± 0.17	0.97 ± 0.38	0.97 ± 0.23
18:0	14.32 ± 1.46	14.53 ± 0.69	13.78 ± 0.70	15.81 ± 0.73	12.72 ± 0.37	15.79 ± 0.56[b]
18:1	15.95 ± 0.09	20.63 ± 1.48[a]	16.48 ± 1.93	12.74 ± 0.33[a]	20.29 ± 4.84	11.74 ± 0.74[a]
18:2	26.22 ± 1.97	27.93 ± 1.03	34.74 ± 2.25	32.33 ± 1.88	33.39 ± 2.74	31.51 ± 0.38
18:3 ω-6	0.28 ± 0.12	0.30 ± 0.16	0.11 ± 0.01	0.32 ± 0.12	0.15 ± 0.11	0.41 ± 0.13
18:3 ω-3	0.76 ± 0.27	0.43 ± 0.06	0.70 ± 0.26	0.58 ± 0.09	0.56 ± 0.08	0.38 ± 0.10
20:3 ω-6	0.73 ± 0.20	0.59 ± 0.06	0.25 ± 0.04	0.26 ± 0.05	0.27 ± 0.13	0.36 ± 0.15
20:4 ω-6	10.53 ± 0.54	9.70 ± 0.58	6.74 ± 0.94	9.74 ± 1.90[b]	6.65 ± 0.74	10.01 ± 1.61[b]
22:4 ω-6	0.95 ± 0.12	0.70 ± 0.06	0.28 ± 0.03	0.60 ± 0.06	0.34 ± 0.16	0.74 ± 0.19
22:5 ω-6	1.28 ± 0.05	1.07 ± 0.10	0.50 ± 0.07	0.68 ± 0.17	0.39 ± 0.14	0.59 ± 0.25
22:5 ω-3	0.92 ± 0.08	1.16 ± 0.09	0.71 ± 0.12	0.93 ± 0.09	0.58 ± 0.07	0.96 ± 0.22
22:6 ω-3	4.84 ± 0.29	4.41 ± 0.29	7.27 ± 0.97	7.73 ± 0.42	6.37 ± 0.47	9.27 ± 0.56[a]
18:1/18:0	1.12 ± 0.10	1.42 ± 0.12[a]	1.19 ± 0.09	0.80 ± 0.02[a]	1.60 ± 0.02	0.76 ± 0.06[a]
Right ventricle						
16:0	22.31 ± 0.71	18.21 ± 1.39	17.61 ± 2.37	18.08 ± 1.70	16.86 ± 0.64	21.29 ± 2.33
16:1	1.25 ± 1.03	1.01 ± 0.27	0.78 ± 0.62	1.03 ± 0.55	0.77 ± 0.25	1.88 ± 0.85
18:0	16.12 ± 0.97	14.14 ± 0.50	12.07 ± 1.30	16.23 ± 1.29[b]	11.74 ± 0.62	16.60 ± 1.46[b]
18:1	13.69 ± 1.14	20.31 ± 0.86[a]	21.30 ± 3.24	13.52 ± 0.57[a]	18.94 ± 3.38	14.38 ± 1.18
18:2	22.04 ± 2.27	26.09 ± 1.65	29.92 ± 1.84	30.80 ± 1.95	34.54 ± 3.42	26.24 ± 3.31[b]
18:3 ω-6	0.34 ± 0.11	0.21 ± 0.13	0.37 ± 0.40	0.38 ± 0.17	0.29 ± 0.11	0.24 ± 0.11
18:3 ω-3	0.31 ± 0.06	0.53 ± 0.22	0.68 ± 0.28	0.50 ± 0.04	0.62 ± 0.17	0.40 ± 0.13
20:3 ω-6	0.65 ± 0.08	0.51 ± 0.04	0.25 ± 0.04	0.30 ± 0.09	0.22 ± 0.09	0.26 ± 0.06
20:4 ω-6	11.69 ± 0.06	9.63 ± 0.30	6.88 ± 0.98	9.41 ± 0.77[b]	6.86 ± 0.36	9.31 ± 0.94[b]
22:4 ω-6	1.04 ± 0.14	0.89 ± 0.16	0.9 ± 0.06	0.82 ± 0.16	1.07 ± 0.34	0.28 ± 0.11
22:5 ω-6	1.22 ± 0.14	1.15 ± 0.14	0.38 ± 0.14	0.49 ± 0.17	0.44 ± 0.05	0.71 ± 0.12
22:5 ω-3	1.14 ± 0.11	1.06 ± 0.06	0.64 ± 0.10	0.84 ± 0.09	0.63 ± 0.17	0.83 ± 0.25
22:6 ω-3	6.97 ± 0.58	4.86 ± 0.66	7.44 ± 1.31	6.90 ± 0.77	6.17 ± 0.48	6.93 ± 0.42
18:1/18:0	0.85 ± 0.08	1.44 ± 0.07[a]	1.79 ± 0.45	0.83 ± 0.04[a]	1.62 ± 0.36	0.87 ± 0.11[a]

Each value was calculated from the results obtained from the analysis of three separate samples. Significantly different from age-matched controls ([a] $P < 0.005$; [b] $P < 0.01$).

CMPH than in controls, whereas the dioleoyl species was more abundant in the heart of healthy controls than in CMPH. The different molecular species composition of the major heart phospholipids in CMPH and in controls was paralleled by a different fatty acid composition of the whole cardiac lipids (**Table 2**). Indeed, the percent content of oleic acid is higher in both right and left ventricles from 20-day-old CMPH than in age-matched controls, while the contrary was evident in 90- and 150-day-old animals. In fact, during the first 2 months after weaning, the percent content of oleic acid decreased by more than 30% in the heart lipids from CMPH, whereas it was constant or even increased in the ventricles of healthy controls. On the other hand, the percent content of stearic acid in the same lipids decreased with age in controls, but slightly increased in CMPH. As a consequence, the oleate-to-stearate ratio decreased in the cardiac lipids of the CMPH while increasing in controls, during development. Moreover, the percent content of arachidonate and docosahexaenoate in the cardiac lipids of 90- and 150-day-old animals was significantly higher in CMPH than in controls. The fatty acid composition of the lipids from other tissues of 3-month-old CMPH was also different from that of age-matched healthy controls. In fact, in all the other tissues analyzed, except for the blood plasma, the oleate-to-stearate ratio is significantly lower in CMPH than in healthy Syrian hamsters (**Table 3**). In particular, oleic acid, which accounts for about 16% of total fatty acids in the livers of healthy hamster, represented only about 11% of the fatty acids contained in the same tissue of CMPH. The low oleate content of CMPH liver lipids was counterbalanced by a higher percent content of stearic and linoleic acids. The analytical data summarized in Fig. 1 and in Tables 1–3 suggest that CMPH, in addition to the described physiopathological disorders of skeletal and cardiac muscle, were affected by a generalized impairment of lipid metabolism. To verify the feasibility of this hypothesis, the pathways involved in the biosynthesis of glycerolipids were examined in vivo by monitoring the hepatic utilization of labeled glycerol and acetate for lipid synthesis.

Incorporation of labeled glycerol into liver lipids

Twenty-day-old control and CMPH were injected intraperitoneally with [2-^{3}H]glycerol. Thirty minutes after the injection, livers from cardiomyopathic (1.73 ± 0.32 g) and control (2.55 ± 0.41 g) hamsters were excised and lipids were extracted and isolated chromatographically.

The incorporation per gram of tissue of the labeled

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TABLE 3. Molar percent composition of individual fatty acids obtained by trans-esterification of whole lipids from various tissues of cardiomyopathic UM-X7.1 and healthy Syrian hamsters

	Plasma		Kidney		Skeletal Muscle		Lung		Liver		Brain	
	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1	UM-X7.1	Control
16:0	27.23 ± 1.11	26.27 ± 1.73	20.03 ± 0.50	18.15 ± 0.39	25.16 ± 2.02	22.07 ± 0.36	37.52 ± 4.47	37.55 ± 2.27	22.06 ± 0.46	21.97 ± 0.67	26.68 ± 0.95	25.64 ± 1.31
16:1	Traces	Traces	1.69 ± 1.12	1.12 ± 0.03	4.66 ± 0.72	2.64 ± 0.31[a]	5.35 ± 0.35	4.20 ± 0.32	Traces	Traces	Traces	Traces
18:0	9.96 ± 0.38	10.79 ± 0.45	15.87 ± 1.45	18.97 ± 0.41	7.36 ± 1.18	8.82 ± 0.84	10.12 ± 1.52	13.12 ± 0.82[b]	15.44 ± 0.94	18.58 ± 0.76[a]	16.24 ± 0.37	18.47 ± 0.69[b]
18:1	21.15 ± 1.57	19.66 ± 1.12	17.44 ± 1.52	12.15 ± 0.21[a]	24.71 ± 1.98	21.84 ± 0.48[b]	20.42 ± 1.21	17.53 ± 1.15[a]	15.97 ± 1.46	10.99 ± 0.61[a]	24.70 ± 1.63	21.27 ± 1.25[b]
18:2	29.87 ± 0.25	32.19 ± 0.78[b]	17.96 ± 0.45	17.59 ± 0.42	27.42 ± 0.89	30.45 ± 1.79[b]	13.14 ± 2.50	13.86 ± 1.40	24.62 ± 1.06	24.35 ± 1.26	1.04 ± 0.39	1.23 ± 0.26
18:3 ω-3	2.16 ± 0.13	3.03 ± 0.76	0.62 ± 0.27	0.46 ± 0.21	1.09 ± 0.39	2.12 ± 0.01[a]	0.74 ± 0.28	0.54 ± 0.12	0.69 ± 0.09	0.73 ± 0.01	1.09 ± 0.79	1.24 ± 0.89
20:3 ω-6	0.42 ± 0.11	0.18 ± 0.01	1.10 ± 0.03	1.11 ± 0.03	0.31 ± 0.04	0.32 ± 0.05	0.65 ± 0.01	0.81 ± 0.05	0.1 ± 0.01	0.15 ± 0.02	0.31 ± 0.04	0.38 ± 0.04
20:4 ω-6	3.99 ± 1.40	3.48 ± 1.51	16.57 ± 1.12	21.25 ± 0.35[a]	4.64 ± 1.27	5.58 ± 0.41	6.23 ± 0.12	6.25 ± 0.71	9.57 ± 0.39	11.76 ± 0.90	10.61 ± 0.54	11.02 ± 0.72
22:4 ω-6	0.13 ± 0.04	0.16 ± 0.04	0.34 ± 0.07	0.51 ± 0.21	0.45 ± 0.10	1.00 ± 0.05[a]	1.49 ± 0.06	1.79 ± 0.37	0.22 ± 0.01	0.25 ± 0.06	2.12 ± 0.07	2.28 ± 0.17
22:5 ω-3	Traces	Traces	0.55 ± 0.03	0.42 ± 0.03	0.59 ± 0.13	0.74 ± 0.01	0.98 ± 0.11	0.79 ± 0.07	1.03 ± 0.06	0.85 ± 0.31	0.15 ± 0.11	0.13 ± 0.02
22:6 ω-3	2.41 ± 0.95	1.81 ± 0.90	4.94 ± 0.49	4.68 ± 0.27	2.39 ± 0.51	2.8 ± 0.11	1.74 ± 0.14	1.81 ± 0.34	9.81 ± 0.56	10.05 ± 0.59	13.67 ± 0.28	16.33 ± 0.49[a]
18:1/18:0	2.13 ± 0.33	1.82 ± 0.21	1.20 ± 0.23	0.69 ± 0.13[a]	3.45 ± 0.87	2.50 ± 0.41[b]	1.95 ± 0.44	1.32 ± 0.14[b]	1.05 ± 0.12	0.60 ± 0.05	1.55 ± 0.09	1.15 ± 0.08

Each value was calculated from the results obtained from the analysis of three separate samples. Significantly different from controls ([a] $P < 0.005$; [b] $P < 0.01$).

TABLE 4. Incorporation of labeled glycerol in hepatic lipids

	Control	UM-X7.1
Lipid labeling (μCi/g liver)	1.36 ± 0.18	0.97 ± 0.13[a]
Labeling of individual lipid class (%)		
TG	39.8 ± 4.6	25.0 ± 4.1[a]
DG	13.5 ± 9.8	17.7 ± 3.6[b]
PtdCho	26.9 ± 3.3	31.3 ± 3.4[b]
PtdEtn	16.6 ± 4.2	22.9 ± 2.4[b]
PtdIns	2.1 ± 1.5	2.7 ± 0.6
PtdSer	0.2 ± 0.1	0.3 ± 0.1

[2-^{3}H]glycerol (100 μCi) was injected intraperitoneally in 20-day-old cardiomyopathic UM-X7.1 and healthy Syrian hamsters. Livers were excised 30 min after injection, washed with saline, and weighed. Lipids were extracted according to Folch, Lees, and Sloane-Stanley (10). Total lipid labeling was quantitated by liquid scintillation counting of 10% aliquots of the whole lipid extracts. Lipid chromatographic analyses were performed on 5% aliquots of each extract. The results reported represent the mean ± SD of the data obtained from three separate experiments. TG, triglyceride; DG, diglyceride. Significantly different from controls ([a] $P < 0.005$; [b] $P < 0.01$).

glycerol into the liver lipids of CMPH accounted for about 71% of that found in healthy animals (**Table 4**), most of this difference being due to a strong depression of triglyceride synthesis.

Incorporation of labeled acetate into liver lipids

The utilization of intraperitoneally injected acetate for the hepatic lipid synthesis was evaluated in 20-day-old cardiomyopathic and control hamsters. In the time interval between 10 min and 2 h after injection, the incorporation of the labeled precursor into liver lipids was significantly lower in CMPH than in controls (**Fig. 2**). Also, the heart and plasma lipid labeling was significantly lower in CMPH than in controls, at any time after acetate injection.

The label distribution among the lipid classes from the liver of cardiomyopathic and control hamsters is reported in **Fig. 3**. Ten minutes after isotope injection, triglyceride was the most heavily labeled lipid class in the liver of healthy controls, its labeling decreasing thereafter, in parallel with the increase in phospholipid labeling. The labeling of the same lipid classes in the liver of CMPH was lower than in controls. The labeling of free fatty acids and diacylglycerols was also lower in CMPH than in healthy controls.

On the other hand, during the first hour after isotope injection, the incorporation of labeled acetate into cholesterol was higher in the liver of cardiomyopathic hamsters than in controls.

The label distribution among the fatty acids isolated by RP-HPLC and silver ion chromatography is reported in **Table 5**. The analytical data obtained point out a dramatic depression in acetate utilization for the synthesis of fatty acids in CMPH liver.

In addition, it appears evident from the same data that the 18:1/18:0 labeling ratio was lower in CMPH than in healthy controls, at any time after isotope administration. All together, these findings strongly suggest that both de novo synthesis of fatty acids and Δ^9-desaturase activity were impaired in the liver of CMPH.

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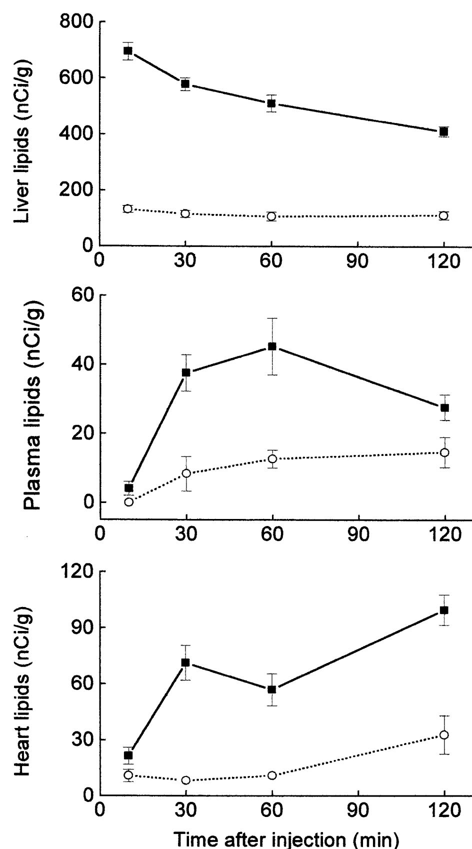

Fig. 2. Incorporation of intraperitoneally injected labeled acetate (30 μCi) in the whole lipids of liver, plasma, and heart from cardiomyopathic (open circles) and healthy (filled squares) Syrian hamsters. Tissues and plasma were kept frozen at −20°C before the analysis. The samples were homogenized in water. Protein content was determined in aliquots of the homogenates. The incorporation of the injected labeled precursor into whole liver, plasma, and heart lipids was assessed by liquid scintillation counting of 10% aliquots of the total lipid extracts. The results are expressed as the amount of radioactive precursor incorporated in the lipids contained in 1 g of tissue.

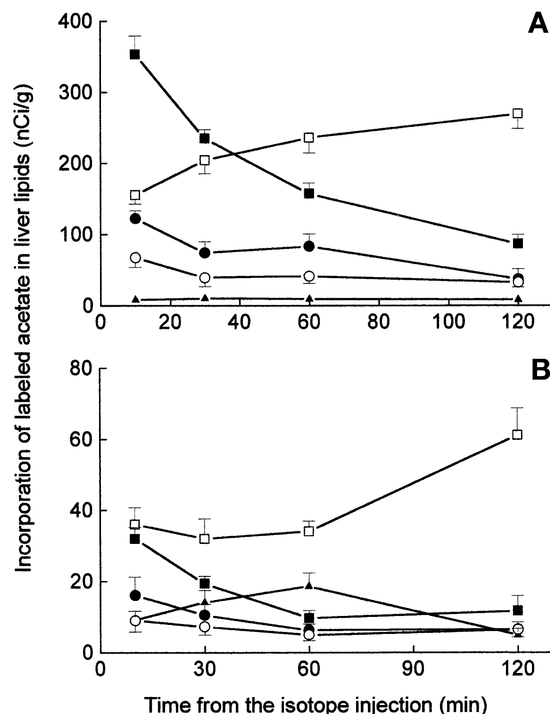

Fig. 3. Time course of liver lipid labeling from intraperitoneally injected acetate. Healthy (A) and cardiomyopathic (B) hamsters were injected intraperitoneally with 30 μCi of [2-^{14}C]acetate. Triglycerides (filled squares), total phospholipids (open squares), unesterified fatty acids (filled circles), diglycerides (open circles), and cholesterol (filled triangles), isolated chromatographically from 10% aliquots of the lipid extracts, were counted for radioactivity.

Quantitation of plasmatic insulin and of mRNAs encoding FAS, SCD, and protein S14

Hepatic RNA was isolated and examined by Northern blot analysis for the levels of mRNAs encoding FAS, SCD, and the "lipogenic" nuclear protein S14. As shown in **Fig. 4**, the content of SCD1 mRNA, of FAS mRNA, and of S14 mRNA in the total liver of CMPH was about 65–70% of that measured in the liver of healthy controls.

Because it has been demonstrated that insulin deficiency or alterations in its signaling pathway are involved in the modulation of the transcription of several genes (3), and that insulin stimulates the expression of SCD1 (7), FAS (19), and S14 (20) in liver, the plasmatic content of insulin was determined in cardiomyopathic and healthy hamsters fed the standard pellet diet. It was found that the plasmatic concentration of insulin in 90-day-old CMPH accounted for only about one fifth of that measured in the plasma of age-matched control animals (**Table 6**), whereas the plasma glucose concentration was not significantly different in the two hamster lines.

Effect of a high carbohydrate diet on lipogenesis

The fact that insulin and dietary carbohydrates are both involved in the transcription of FAS, SCD1, and protein S14 genes prompted us to investigate the effect of different meal-feeding protocols on the expression of the same proteins. Groups of cardiomyopathic and control hamsters were fed for 48 h either with the conventional pellet chow or with a low fat vegetal diet or with a high carbohydrate diet. After dietary conditioning, plasma insulin level and hepatic utilization of intraperitoneally injected labeled acetate were evaluated. In addition, the content of the mRNAs encoding SCD, FAS, and protein S14 in the liver of diet-conditioned animals was quantitated by Northern blot analysis.

With regard to the effect of dietary manipulations on the plasmatic insulin level it was found that the concentration of insulin almost doubled in Syrian hamsters fed the high carbohydrate diet, whereas the increase was much less consistent in CMPH fed the same diet. On the other hand, when fresh vegetables were substituted for the pellet chow, only minor changes in plasma insulin content were induced in both animal strains (Table 6).

The plasma glucose concentration of cardiomyopathic and control hamsters was in the physiological range, after each of the dietary treatments (Table 6).

The level of FAS mRNA and of SCD1 mRNA was strongly increased in the liver of both hamster strains conditioned either with the high carbohydrate diet or with

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TABLE 5. Incorporation of isotopically labeled acetate in the fatty acids of liver lipids

	Time after Isotope Injection (min)							
	Healthy Syrian Hamsters				Cardiomyopathic UM-X7.1 Hamsters			
	10	30	60	120	10	30	60	120
16:0	339.9 ± 20.8	262.8 ± 25.7	250.5 ± 21.9	196.8 ± 5.4	41.1 ± 1.2	25.6 ± 8.2	23.5 ± 2.6	37.8 ± 3.2
16:1	15.9 ± 2.0	12.0 ± 2.5	9.3 ± 3.4	8.8 ± 1.4	3.1 ± 1.6	2.8 ± 1.6	1.4 ± 1.1	0.7 ± 0.4
18:0	83.9 ± 4.7	63.2 ± 6.7	81.8 ± 25.6	90.7 ± 15.4	23.2 ± 0.6	18.4 ± 5.2	16.5 ± 1.4	33.5 ± 1.5
18:1	222.0 ± 26.6	171.8 ± 11.7	136.2 ± 32.2	119.3 ± 15.4	21.6 ± 1.1	14.8 ± 4.3	3.5 ± 0.6	6.4 ± 1.3
20:3	24.0 ± 2.8	18.1 ± 2.4	10.4 ± 2.3	6.3 ± 1.2	6.4 ± 3.5	7.0 ± 3.7	4.2 ± 1.8	1.0 ± 0.4
20:4	Traces	0.6 ± 0.2	1.2 ± 0.7	2.8 ± 1.1	Traces	0.6 ± 0.4	2.2 ± 1.1	3.5 ± 1.3
18:1/18:0	2.65 ± 0.46	2.72 ± 0.61	1.66 ± 0.78	1.32 ± 0.40	0.94 ± 0.08	0.81 ± 0.35	0.24 ± 0.04	0.20 ± 0.01

[2-^{14}C]acetate (30 μCi) was injected intraperitoneally into 20-day-old cardiomyopathic UM-X7.1 and healthy Syrian hamsters. At the stated times after injection, livers were excised and lipids were extracted. After trans-esterification, individual fatty acid methyl esters were separated by RP-HPLC of 20% aliquots of the whole samples. The labeling of individual fatty acid methyl esters (nanocuries per gram of liver) and the oleate-to-stearate ratios were calculated as means ± SD of the results obtained from three separate experiments.

the vegetal diet (Fig. 4). However, after being fed the high carbohydrate diet, the liver of healthy hamsters exhibited a higher content of both mRNAs than that of CMPH. On the other hand, when hamsters were fed with fresh vegetables, livers from cardiomyopathic and control hamsters exhibited comparable levels of FAS mRNA, SCD1 mRNA, and S14 mRNA.

The effect of the different diets on the mRNAs levels was corroborated by the acetate incorporation experiments. In fact, the hepatic utilization of labeled acetate for fatty acid synthesis and elongation was different in the animals conditioned with different diets (**Table 7**). Indeed, when CMPH were fed with the fat-free vegetal diet,

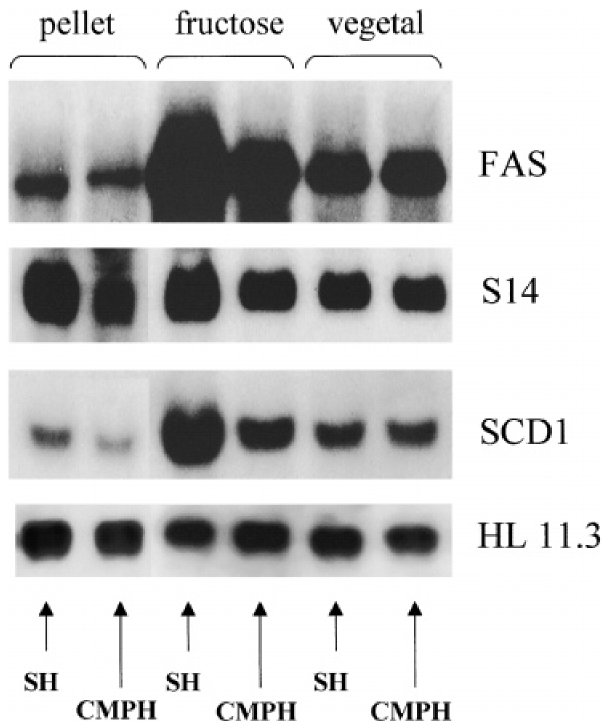

Fig. 4. Northern blot analysis of RNA of liver from cardiomyopathic UM-X7.1 and control hamsters fed different diets. HL11.3 cDNA was used as a probe for checking the integrity and amount of loaded RNAs. SH, Syrian hamster.

the de novo synthesis of saturated and Δ^9-monoenoic acids was almost tripled, when compared with animals fed the pellet chow. On the other hand, the label incorporation in the liver lipids of healthy controls was not significantly affected by the same dietary change.

As expected, the high carbohydrate diet induced an impressive increase in the FAS activity both in cardiomyopathic and healthy hamsters. However, the fatty acid synthesis of healthy hamsters was much more stimulated than that of cardiomyopathic animals by dietary fructose.

Moreover, in accordance with the results of the Northern blot analysis, the high fructose diet resulted in an increase in the rate of desaturation of stearate to oleate both in cardiomyopathic UM-X7.1 and healthy Syrian hamsters (Table 7).

When cardiomyopathic UM-X7.1 hamsters were fed with the low fat, high carbohydrate diet, the Δ^9-desaturase activity increased by about two times, as compared with that measured in the liver of pellet-fed hamsters. On the other hand, the Δ^9-desaturase activity of healthy hamsters was not significantly modified by the same dietary change.

DISCUSSION

In the present work, starting from preliminary observation on the lipid composition of the cardiac tissue, evidence is given for a generalized disturbance of lipid metabolism in the cardiomyopathic UM-X7.1 hamster.

The phospholipid-to-protein ratio of cardiac ventricles from 20-day-old CMPH was not significantly different from that of age-matched healthy controls. A relevant change in the same ratio occured after weaning, as animals started to eat the standard pellet chow.

It is worth mentioning that, because of its more intense mechanical work, *a*) the phospholipid-to-protein ratio is low in the left ventricle, as compared with the right ventricle, at any age, and *b*) during development, the same ratio decreases more conspicuously in the left than in the right ventricle. In this last regard, it appears relevant that, during development, the lessening of the phospholipid content per milligram protein in both ventricles of CMPH was higher than that occurring in healthy hamsters.

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TABLE 6. Plasma insulin and glucose content of cardiomyopathic UM-X7.1 and healthy controls fed different diets

	Pellet		High Carbohydrate		Fresh Vegetables	
	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1
Insulin (ng/ml)	1.25 ± 0.16	0.25 ± 0.05[a]	2.50 ± 0.31	0.37 ± 0.06[a]	1.31 ± 0.14	0.44 ± 0.07[a]
Glucose (mg/dl)	96.5 ± 4.7	105.0 ± 7.5	128.0 ± 8.7	95.5 ± 6.4[b]	118.0 ± 8.5	85.5 ± 6.8[b]

Two groups of 90-day-old cardiomyopathic and control hamsters were fed standard pellet chow. Other groups of age-matched control and cardiomyopathic hamsters received either a fat-free high carbohydrate (80% fructose) diet or a fat-free vegetal diet for 48 h before drawing of blood for quantitating insulin and glucose. The data reported were calculated as means ± SD of the results obtained from three separate determinations. Significantly different from controls feeding the same diet ([a] $P < 0.005$; [b] $P < 0.001$).

Other dissimilarities between the two hamster groups were put into evidence by analyzing the fatty acid composition of ventricular lipids. In fact, it was observed that, in the time interval between weaning and the fifth month of age, the percent content of oleic acid decreased in the ventricular lipids of CMPH whereas it increased in controls. In parallel, the percent content of arachidonate decreased in the lipids from healthy hamster ventricles whereas it did not change in CMPH. Because of these age-related changes, the whole heart phosphatidylcholine of adult CMPH was significantly depleted of dioleoyl molecular species whereas both phosphatidylcholine and phosphatidylethanolamine were enriched in 1-stearoyl-2-arachidonoyl molecular species.

All together, these analytical data are consistent with the hypothesis that the CMPH is characterized by a disturbance of lipid metabolism. An indirect indication that such an impairment is generalized and not restricted to the heart is derived from evidence that the fatty acid composition of the lipids extracted from other tissues was also different in healthy and cardiomyopathic hamsters.

Taking into consideration the fact that most lipid synthesis takes place in liver, experiments were devised to quantify the efficiency of the metabolic routes leading to the synthesis of glycerolipids and of their constituent fatty acids in liver.

The main results obtained from these experiments can be summarized as follows: *a*) The incorporation of intraperitoneally injected [2-^{3}H]glycerol and [2-^{14}C]acetate into liver lipids was low in CMPH, as compared with healthy controls; *b*) the rate of Δ^9-desaturation of saturated fatty acids was heavily depressed in CMPH; and *c*) the hepatic content of both FAS mRNA and SCD1 mRNA was lower in CMPH than in healthy controls.

The metabolic data obtained and the low hepatic content of the mRNAs encoding the enzymatic proteins involved appear to be a direct consequence of the reduced plasmatic level of insulin in CMPH. Indeed, it is known (3, 4) that the expression of glycerol phosphate acyltransferase, FAS, and SCD, in liver is positively modulated by carbohydrates and insulin, while being depressed by polyenoic fatty acids. Therefore, because both control and CMPH were fed the same diet, the reduced activity of the above-mentioned enzymes in CMPH appears to be a consequence of the low level of circulating insulin.

As expected, when the diet was turned from the conventional laboratory pellet to a high carbohydrate fat-free diet, both FAS and SCD1 mRNA content increased in car-

TABLE 7. Effect of diet on acetate incorporation in liver lipids

	Pellet		Fructose		Vegetal	
	Control	UM-X7.1	Control	UM-X7.1	Control	UM-X7.1
16:0	110.2 ± 5.3	34.4 ± 5.6	1018.5 ± 99.2	538.3 ± 35.3	110.5 ± 11.3	258.6 ± 28.1
16:1	4.1 ± 1.2	1.3 ± 0.4	172.4 ± 13.2	88.9 ± 11.5	3.2 ± 0.7	9.2 ± 1.8
18:0	33.1 ± 3.8	22.3 ± 3.4	226.7 ± 22.1	144.7 ± 10.3	28.3 ± 4.2	70.2 ± 7.7
18:1	26.6 ± 5.2	7.7 ± 0.8	918.7 ± 73.6	267.0 ± 17.5	19.1 ± 3.2	44.3 ± 6.0
20:3 (ω-6)	2.6 ± 0.4	6.6 ± 2.2	ND	ND	5.0 ± 1.4	10.3 ± 2.5
20:4 (ω-6)	0.8 ± 0.5	3.4 ± 1.5	ND	ND	1.7 ± 0.7	3.2 ± 1.4
18:1/18:0	0.80 ± 0.12	0.35 ± 0.10[a]	4.05 ± 0.21	1.84 ± 0.08[a]	0.66 ± 0.07	0.64 ± 0.16

After 12 h of fasting, 3-month-old hamsters were fed for 48 h with different diets (see text for details). [2-^{14}C]acetate was injected intraperitoneally into cardiomyopathic UM-X7.1 and healthy Syrian hamsters. Fifteen minutes after injection, livers were excised (7.7 ± 0.71 g, control hamsters; 4.33 ± 0.46 g, cardiomyopathic hamsters). Hepatic lipids were extracted and fatty acids were converted into the respective methyl esters. Twenty percent aliquots of the whole samples were subjected to RP-HPLC. The labeling of individual fatty acid methyl esters (nanocuries per gram of liver) and the oleate-to-stearate ratios were calculated as means ± SD of the results obtained from three separate experiments. ND, not detected.
[a] $P < 0.005$.

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diomyopathic and healthy hamsters. As a consequence, the same dietary change induced an increase in FAS and SCD activities both in CMPH and in controls.

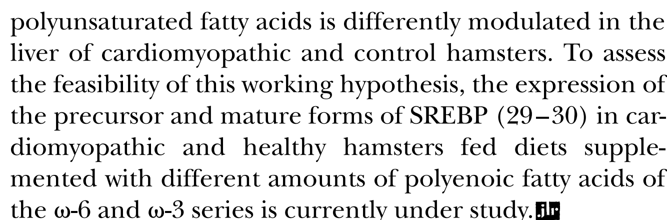
However, after the dietary change, the hepatic FAS mRNA and SCD1 mRNA levels were still higher in healthy hamsters that in CMPH. As a consequence, the expression of hepatic FAS and SCD was also different in the two groups of animals, as judged by their activities in comparison with fructose-fed animals. This finding was not unexpected, considering previous evidences reported by several authors. In fact, Waters and Ntambi (7) demonstrated that, when separately administered to streptozotocin-induced diabetic rats, insulin and dietary carbohydrates produce an increase in hepatic SCD1 mRNA, but a combination of both hormonal and nutritional factors is needed to obtain a full induction of gene expression. The same consideration applies to the stimulation exerted by insulin and dietary carbohydrates on the expression and activity of FAS (21, 22).

In both cases, because of the dependence of carbohydrate metabolism on insulin, it is not easy to outline the role independently played by the two effectors. Indeed, insulin could act as a modulator of gene expression either by increasing the cytoplasmatic concentration of a specific metabolite of carbohydrates or through a carbohydrate-independent mechanism (23, 24). The results reported in the present article do not provide any new insight in this context and could be explained by either one of the two hypotheses.

In addition to the different responsiveness of lipogenic enzymes to dietary carbohydrates in cardiomyopathic and healthy hamsters, FAS and SCD1 appear to be differently modulated also by dietary lipids in the two hamster strains. In fact, when the diet was turned from conventional pellets to a fat-free vegetal diet, the incorporation of acetate in fatty acids did not change significantly in the liver of healthy controls whereas it increased five times in cardiomyopathic animals.

A different response to the same dietary change was observed also when the activity of SCD1 was taken into consideration. Indeed, after the dietary change, SCD1 activity did not change significantly in controls whereas it was almost doubled in the cardiomyopathic animals. In this context, it is worth mentioning that cardiomyopathic and control hamsters fed the fat-free vegetal diet exhibited comparable SCD activity and SCD1 mRNA content in liver, although the level of circulating insulin was still different in the two hamster strains.

It has been shown previously (25) that dietary polyunsaturated fatty acids (PUFA) induce the expression of the enzymes involved in hepatic lipolysis through a peroxisome proliferator-activated receptor α (PPARα)-mediated mechanism, whereas PPARα is not involved in the PUFA-mediated inhibition of FAS, SCD1, and S14, the transcription of which is under the control of the sterol regulatory element-binding proteins (SREBP) (26–28). If this fact is taken into consideration, the results reported in the present work could raise the hypothesis that the cascade of events that occur in response to the dietary intake of



polyunsaturated fatty acids is differently modulated in the liver of cardiomyopathic and control hamsters. To assess the feasibility of this working hypothesis, the expression of the precursor and mature forms of SREBP (29–30) in cardiomyopathic and healthy hamsters fed diets supplemented with different amounts of polyenoic fatty acids of the ω-6 and ω-3 series is currently under study.

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