

# Impaired lipid metabolism in cardiomyopathic hamsters

Alba Vecchini, Luciano Binaglia, Marilena Minieri\*, Paolo Di Nardo\*

Department of Internal Medicine and Endocrine-Metabolic Sciences, University of Perugia, Perugia,

\*Department of Internal Medicine, Laboratory of Molecular Cardiology, Tor Vergata University of Rome, Rome, Italy

(Ital Heart J 2000; 1 (Suppl 3): S22-S24)

Research funded by  
Agenzia Spaziale Italiana.

Address:

Prof. Luciano Binaglia

Dipartimento di Medicina  
Interna e Scienze  
Endocrine e Metaboliche  
Università degli Studi  
Via del Giochetto, 3  
06126 Perugia  
E-mail:  
binaglia@unipg.it

## Introduction

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. Recent studies have demonstrated that the primary cause of the UM-X7.1 hamster cardiomyopathy is represented by a mutation of the gene encoding  $\delta$ -sarcoglycan<sup>1</sup>. In the present work evidence is given that, as a consequence of its low plasma insulin level, the CMPH is also affected by a generalized impairment of lipid metabolism and that dietary manipulations are able to correct such a metabolic disturbance.

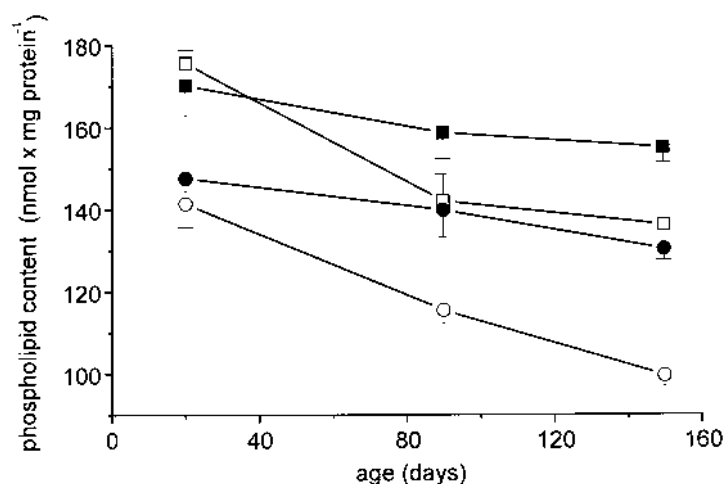
Indeed it is known that, in addition to its function in modulating the intracellular metabolism, the major role of insulin is represented by its involvement in the regulation of the expression of a wide series of genes<sup>2</sup>,

comprising acetyl-CoA carboxylase, fatty acid synthase (FAS), stearoyl-CoA desaturase (SCD), *sn*-glycerol-3-phosphate acyltransferase and the nuclear lipogenic protein S14.

## Experimental studies

**Lipid composition of cardiac tissue.** The phospholipid content of cardiac ventricles from 20 day-old CMPHs (line UM-X7.1) is not significantly different from that of age-matched healthy controls whereas a relevant change in the same ratio occurs after weaning, as animals are fed the standard pellet chow (Fig. 1).

It is noteworthy to mention that, due to its more intense mechanical work, the phospholipid to protein ratio is low in the left ventricle, compared to the right one, at any age. During development, the same ratio de-



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

creases more consistently in the left than in the right ventricle. Therefore, it appears relevant that, during development, the lessening of the phospholipid content per milligram protein in both ventricles of CMPHs is higher than that occurring in healthy hamsters.

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 life, the percent content of oleic acid decreases by about 9% in the ventricular lipids of CMPHs whereas it increases by about 4.5% in controls. In parallel, the percent content of arachidonate decreases significantly in the lipids from healthy hamster ventricles while it does not change in CMPHs. 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 derives from the evidence that also the fatty acid composition of the lipids extracted from other tissues is different in healthy and CMPHs (not shown).

**Impairment of hepatic lipid metabolism in cardiomyopathic hamsters.** Taking into consideration the fact that most of the lipid synthesis takes place in the 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 the liver.

The main results obtained from these experiments can be summarized as follows:

the incorporation of intraperitoneally-injected [ $^3\text{H}$ ]glycerol and [ $^{14}\text{C}$ ]acetate into the liver lipids is low in CMPHs, as compared to healthy controls (Table I);

the rate of  $\Delta^9$ -desaturation of saturated fatty acids is heavily depressed in CMPHs (Table II);

the hepatic content of both FAS mRNA and SCD1 mRNA is lower in CMPHs than in healthy controls (Fig. 2).

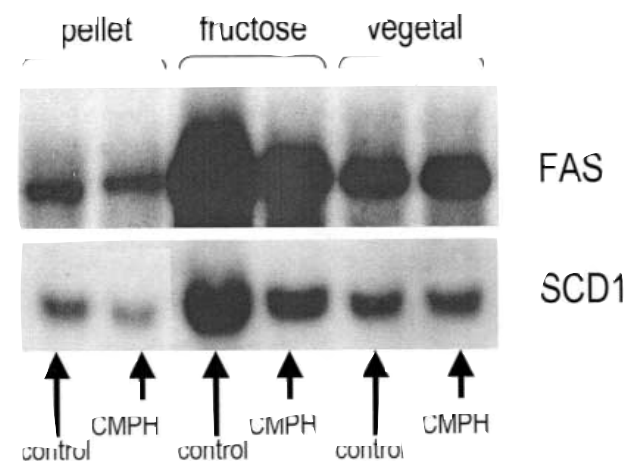
The metabolic data obtained and the low hepatic content of the mRNAs encoding the enzymatic proteins involved appear to be a consequence of the low plasma insulin level in CMPHs (0.25 – 0.05 ng/ml) as compared to the plasma insulin level of healthy controls

(1.25 – 0.16 ng/ml). Indeed, it is known<sup>2,3</sup> that the expression of a series of hepatic lipogenic enzymes is positively modulated by carbohydrates and insulin, while being depressed by polyenoic fatty acids. Therefore, since both controls and CMPHs were fed the same diet, the low level of circulating insulin could explain the reduced expression of the above-mentioned enzymes.

**Effect of nutrients on the expression and the activity of lipogenic enzymes.** As expected, when the diet was changed from the conventional laboratory pellet to a high-carbohydrate fat-free diet (80% fructose, 20%

**Table I.** Incorporation of intraperitoneally-injected lipid precursors into liver lipids of 90-day-old hamsters.

Intraperitoneally-injected lipid precursor	Incorporation into liver lipids ( $\mu\text{Ci}$ )	
	Cardiomyopathic hamsters	Healthy Syrian hamsters
[ $^3\text{H}$ ]glycerol (30 $\mu\text{Ci}$ )	1.98 – 0.26	3.45 – 0.51
[ $^{14}\text{C}$ ]acetate (100 $\mu\text{Ci}$ )	0.33 – 0.08	1.36 – 0.30



**Figure 2.** Northern blot analysis of RNA of the liver from cardiomyopathic (CMPH) and control hamsters fed with different diets. HL11.3 cDNA, used as a probe for checking integrity and amount of loaded RNAs, gave comparable signals in the different lanes (not shown). FAS = fatty acid synthase; SCD = stearoyl-CoA desaturase.

**Table II.** Incorporation of intraperitoneally-injected acetate into liver fatty acids.

	Pellet		Fructose		Vegetal diet	
	CMPHs	Controls	CMPHs	Controls	CMPHs	Controls
FAS (nCi)	280.2	1320.5	1611.4	1212.2	4656.7	17895.4
SCD (18:1/18:0)	0.34	0.81	1.82	4.12	0.64	0.66

FAS = fatty acid synthase; SCD = stearoyl-CoA desaturase. [ $^{14}\text{C}$ ]-labeled acetate (30  $\mu\text{Ci}$ ) was injected intraperitoneally in 90-day-old cardiomyopathic hamsters (CMPHs) and age-matched healthy controls. Livers were excised after 15 min from the isotope injection. Fatty acid methyl esters were obtained by transesterification of the whole lipid extract and isolated by reverse-phase high-performance liquid chromatography. The FAS activity was evaluated measuring the radioactivity incorporated in saturated and monoenoic fatty acids. The labeling ratio 18:1/18:0 was taken as a measure of SCD activity. The data shown in the present table were obtained from one out of four similar experiments.

fiber), both FAS and SCD1 mRNA content increased in cardiomyopathic and healthy hamsters (Fig. 2). As a consequence, the same dietary change induced an increase in FAS and SCD activities both in CMPHs and controls (Table II).

However, after the dietary change, the hepatic FAS mRNA and SCD1 mRNA levels were still higher in healthy hamsters than in CMPHs. As a consequence, also the expression of hepatic FAS and SCD was different in the two groups of animals, as judged by their activities in fructose-fed animals. This finding was not unexpected, since it had previously been shown that a full induction of both FAS and SCD expression results only from the combined action of hormonal and nutritional factors<sup>4-6</sup>.

In addition to the different responsiveness of lipogenic enzymes to dietary carbohydrates in cardiomyopathic and healthy hamsters, FAS and SCD appear to be differently modulated also by dietary lipids in the two hamster strains. In fact, when the diet was changed from the conventional pellet 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 5 times in cardiomyopathic animals.

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

## Conclusions

It has previously been shown<sup>7</sup> that dietary polyunsaturated fatty acids induce the expression of the enzymes involved in hepatic lipolysis through a PPAR $\alpha$ -mediat-

ed mechanism whereas PPAR $\alpha$  is not involved in the polyunsaturated fatty acid-mediated inhibition of FAS, SCD1 and S14, whose transcription is under the control of sterol regulatory element-binding proteins<sup>8-10</sup>. If this fact is taken into consideration, the results reported in the present work could raise the hypothesis that the cascade of events occurring in response to the dietary intake of polyunsaturated fatty acids is differently modulated in the liver of cardiomyopathic and control hamsters.

## References

1. Nigro V, Okazaki Y, Belsito A, et al. Identification of the Syrian hamster cardiomyopathy gene. *Hum Mol Genet* 1997; 6: 601-7.
2. O'Brien RM, Granner DK. Regulation of gene expression by insulin. *Biochem J* 1991; 278: 609-19.
3. O'Brien RM, Granner DK. Regulation of gene expression by insulin. *Physiol Rev* 1996; 76: 1109-61.
4. Waters KM, Ntambi JM. Insulin and dietary fructose induce stearoyl-CoA desaturase 1 gene expression in liver of diabetic mice. *J Biol Chem* 1994; 269: 27773-7.
5. Girard J, Ferre P, Foufelle F. Mechanisms by which carbohydrates regulate expression of genes for glycolytic and lipogenic enzymes. *Annu Rev Nutr* 1997; 17: 325-52.
6. Casado M, Vallet VS, Kahn A, Vaulont S. Essential role in vivo of upstream stimulatory factors for a normal dietary response of the fatty acid synthase gene in the liver. *J Biol Chem* 1999; 274: 2009-13.
7. Clarke SD, Jump DB. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu Rev Nutr* 1994; 14: 83-98.
8. Ren B, Thelen AP, Peters JM, Gonzales FJ, Jump DB. Polyunsaturated fatty acid suppression of hepatic fatty acid synthase and S14 gene expression does not require peroxisome proliferator-activated receptor  $\alpha$ . *J Biol Chem* 1997; 272: 26827-32.
9. Kim HJ, Takahashi M, Ezaki O. Fish oil feeding decreases mature sterol regulatory element-binding protein 1 (SREBP-1) by down-regulation of SREBP-1c mRNA in mouse liver. *J Biol Chem* 1999; 274: 25892-8.
10. Foretz M, Guichard C, Ferre P, Foufelle F. Sterol regulatory element binding protein-1c is a major mediator of insulin action on the hepatic expression of glucokinase and lipogenesis-related genes. *Proc Natl Acad Sci USA* 1999; 96: 12737-42.