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Effects of *L*-Carnitine on Sucrose-Induced Hyperlipidaemia

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Summary. *L*-Carnitine, *L*-(−)-β-hydroxy-γ-trimethylaminobutyrate, plays an important role as a factor necessary for the transport of long-chain fatty acids into the mitochondria. In order to investigate the influence of *L*-carnitine on hyperlipidaemias, the experimental model of the sucrose-induced hypertriglyceridaemia of the rat was used. In these experiments *L*-carnitine in the dose of 11 mg per day and 100 g body weight (over the period of 1 week) was able to antagonize the sucrose-induced hypertriglyceridaemia and the increase of serum free fatty acid level in female rats of the Wistar strain. Carnitine administration did not change the activities of lipogenic enzymes and fatty acid synthesis in the liver. However, *L*-carnitine increases the rate of hepatic fatty acid oxidation. Our results indicate a hypotriglyceridemic and free fatty acid lowering effect of *L*-carnitine, and suggest the use of this compound in the therapy of hyperlipidaemias.

Keywords. *L*-Carnitine; Sucrose; Free fatty acids; Triglycerides; Lipogenic enzymes; Fatty acid synthesis; Fatty acid oxidation.

Introduction

L-Carnitine, *L*-(−)-β-hydroxy-γ-trimethylaminobutyrate, plays an important role in fatty acid metabolism. The main biological function of carnitine is the transport of fatty acids through the inner mitochondrial membrane to sites of β-oxidation in the mitochondrial matrix.

Various investigations have clearly demonstrated that carnitine deficiency occurs both in classical genetic human carnitine deficiencies and in a number of other commonly occurring diseases [1–4]. Some findings suggested that carnitine may be useful not only in the treatment of hypertriglyceridaemia and muscle carnitine deficiency induced during haemodialysis in uraemic patients [5–7], but also in other hyper- and dyslipidemic disorders [8–10]. Because of its function in fatty acid metabolism, carnitine has been considered as therapeutic agent in the

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treatment of hyperlipidaemias. Clinical investigations indicate that carnitine may be useful in the treatment of hyperlipoproteinaemias.

Marked effects in hypertriglyceridemic patients were observed [9–12]. Several animal models of hyperlipoproteinaemia were used to clarify mechanisms of carnitine effects on lipoprotein metabolism. It is speculated that carnitine administration, by promoting beta-oxidation, lowers synthesis of very low density lipoproteins in rat fed olive oil [13, 14]. In this animal model carnitine significantly decreased the concentrations of triglycerides, cholesterol, phospholipids, and free fatty acids in plasma. In rats administered with a 30% corn oil diet, simultaneous application of carnitine reduced serum triglyceride and cholesterol concentrations. Furthermore the lipid concentrations in liver were lowered under the conditions of high-fat diet [15]. Experimental investigations with rat hepatocyte cells indicated that *L*-carnitine affects the cholesterol metabolism through an inhibition of 3-hydroxy-3-methyl-glutaryl-CoA reductase activity concomitant with an increased (125J) LDL binding to the hepatocytes [16, 17]. In rabbits as a good model of diet induced hyperlipoproteinaemia early atherosclerotic lesions are developed. It was seen that administration of *L*-carnitine to rabbits fed a high fat-diet decreased plasma cholesterol and triglyceride levels and reduced the extent of liver steatosis. A lowering of total plasma triglycerides, very low density lipoprotein triglycerides and intermediate density lipoprotein triglycerides under the influence of propionyl-*L*-carnitine treatment was also observed in rabbits that received a hyperlipidemic cholesterol-rich diet. These investigations also showed that long-term oral propionyl-*L*-carnitine administration was associated with a reduction of plaque cell proliferation and a reduction of the severity of aortic atherosclerotic lesions [18, 19].

In order to investigate the influence of carnitine on hypertriglyceridemias the experimental model of carbohydrate-induced hypertriglyceridaemia was used, especially the sucrose-induced hyperlipoproteinaemia. It was the aim of the present investigations to study the effects of *L*-carnitine on parameters of lipid metabolism in serum on the activities of hepatic lipogenic enzymes, on fatty acid synthesis and on hepatic fatty acid oxidation in normal and sucrose-fed female Wistar rats.

Results and Discussion

A high intake of dietary sucrose leads to a significant increase of plasma triglyceride and free fatty acid concentrations. The hyperlipidemic effect of sucrose is associated with the intense conversion of fructose to fatty acids and triglycerides in the liver. It has been shown that dietary sucrose increases the activities of several lipogenic liver enzymes, among others citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase. Therefor, the sucrose-rich alimented rat is a good model with respect to the endogenous carbohydrate-induced hypertriglyceridaemia [21, 22]. This model was used to test mechanisms of the influence of carnitine on blood lipid levels. In view of the question whether exogenous carnitine does not only effect fatty acid oxidation but also other metabolic pathways, the activities of lipogenic liver enzymes and the rate of hepatic fatty acid synthesis were measured.

The administration of *L*-carnitine does not change the basal serum triglyceride levels significantly. Under the conditions of the sucrose-rich diet the well-known

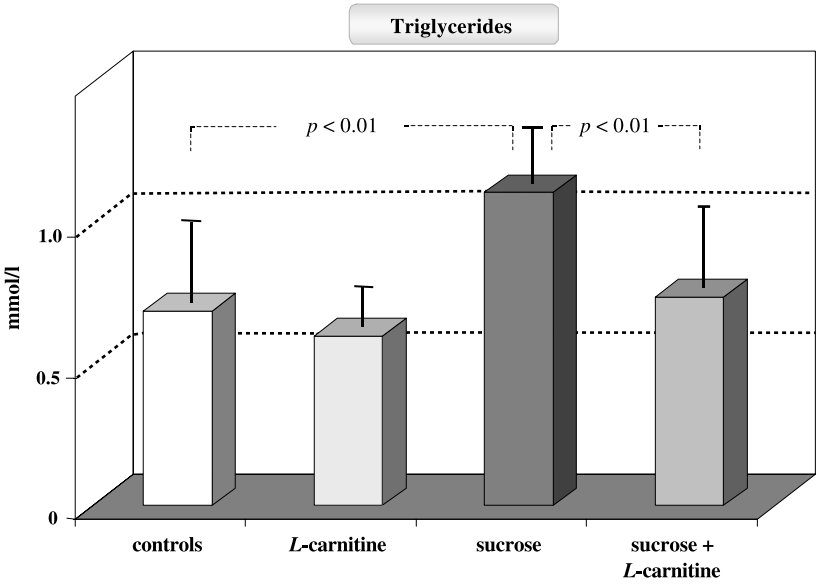

Fig. 1. Serum triglyceride levels under the influence of sucrose-rich diet and *L*-carnitine ($n = 36$ female rats of the Wistar strain in each experimental group, average values ± standard error, p = probability of error of the significance)

increase of serum triglyceride levels can be seen. *L*-Carnitine antagonizes this sucrose effect (Fig. 1).

The *L*-carnitine supplementation does not change the basal serum free fatty acid levels significantly. The effect of carnitine with and without sucrose-rich diet

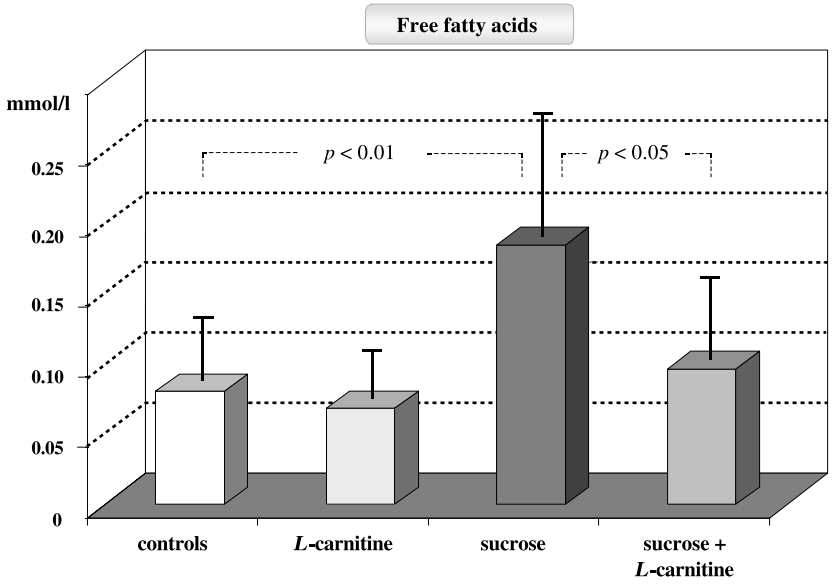

Fig. 2. Serum free fatty acid concentration under the conditions of sucrose-rich diet and *L*-carnitine ($n = 15$ female rats of the Wistar strain in each experimental group, average values ± standard error, p = probability of error of the significance)

on the level of free fatty acids is shown in Fig. 2. Serum free fatty acid concentrations in the carnitine plus sucrose group were significantly lower ($p<0.05$) when compared with the sucrose-rich diet group.

Furthermore, the activities of the lipogenic liver enzymes, citrate cleavage enzyme, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate-dehydrogenase were determined in order to study a possible effect of carnitine on these enzymes. Carnitine does not change the activities of these lipogenic enzymes. Under the conditions of a sucrose-rich diet the activities of these lipogenic enzymes increased two-fold, but carnitine had no further effect (Figs. 3 and 4).

Under the conditions of sucrose-rich diet not only the activities of lipogenic liver enzymes are elevated but also the rate of fatty acid synthesis was studied

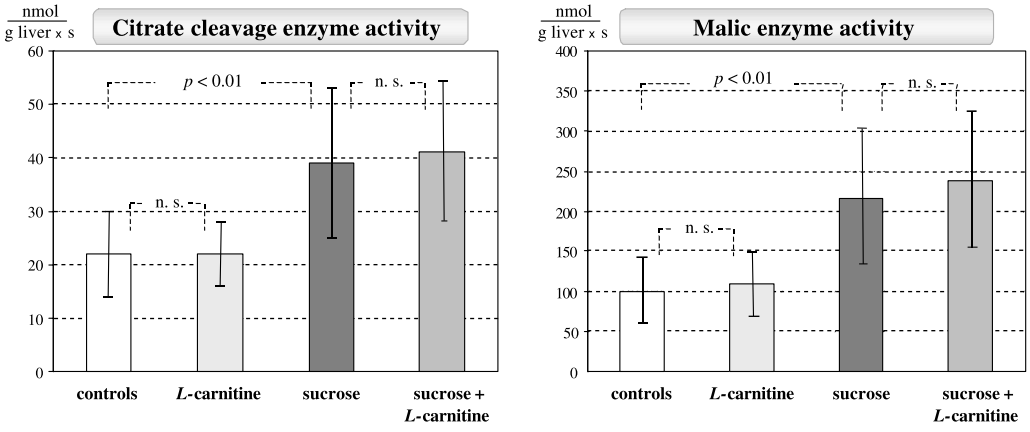

Fig. 3. Activities of lipogenic liver enzymes (citrate cleavage enzyme and malic enzyme) and the influence of sucrose-rich diet and *L*-carnitine (mean ± standard error, number of the animals in the experimental groups $n = 36$)

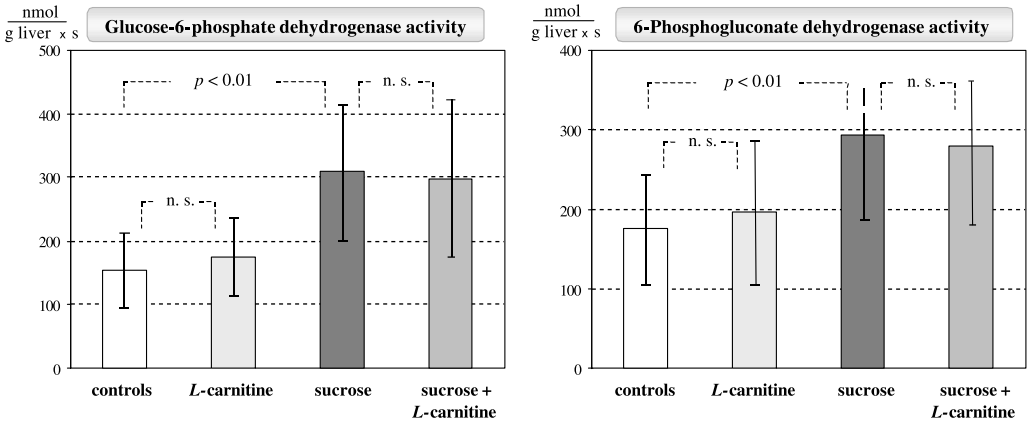

Fig. 4. Activities of lipogenic liver enzymes (glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase) and the influence of sucrose-rich diet and *L*-carnitine (mean ± standard error, number of the animals in the experimental groups $n = 36$)

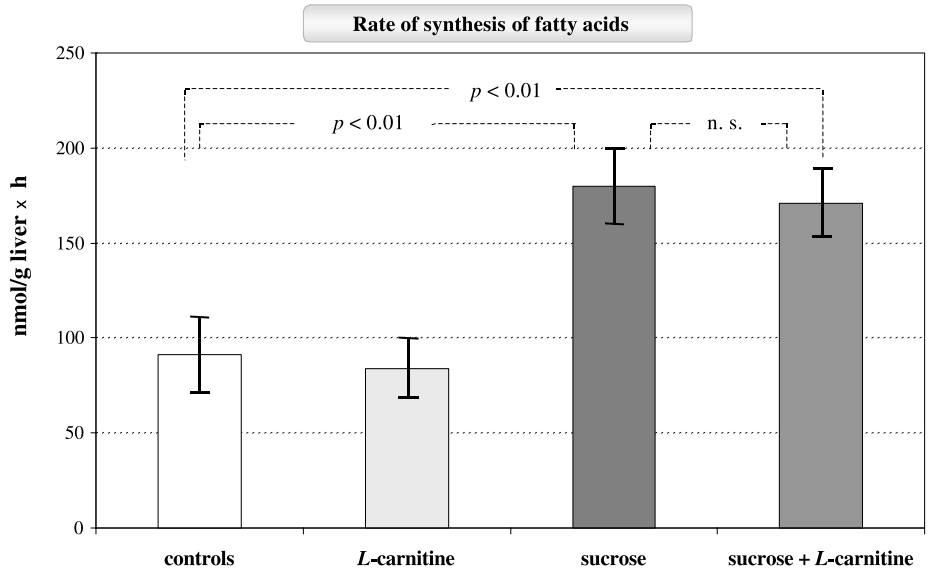

Fig. 5. Synthesis of fatty acid in liver palmitic acid (*PA*) equivalents – under the effects of sucrose rich diet and carnitine (mean ± standard error, $n = 23$ of animals in each group)

(Fig. 5). Sucrose feeding results in a significant increase of the rate of fatty acid synthesis. However, carnitine has no effect on sucrose induced elevation of fatty acid synthesis.

The increase of activities of lipogenic liver enzymes and the rise in velocity of fatty acid synthesis under the influence of sucrose-rich diet leads to the hypertriglyceridaemia in the rat. It can be concluded that carnitine administration does not change the lipogenic state of the liver.

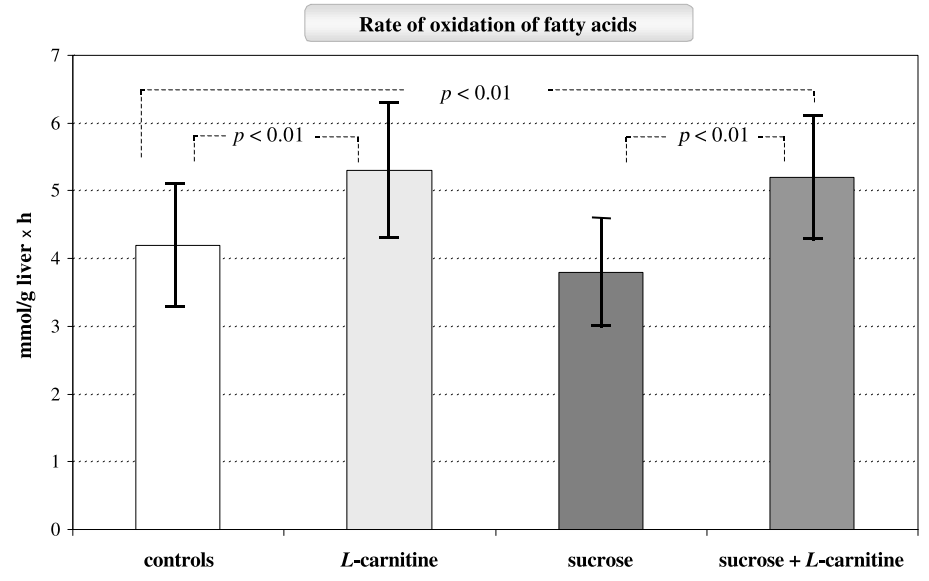

Fig. 6. Oxidation of palmitate of liver-[^{14}C] CO_2 production – under the effect of sucrose rich diet and *L*-carnitine (mean ± standard error, $n = 23$ of animals in each group)

Carnitine increases the rates of hepatic fatty acid oxidation ($p < 0.01$). An elevated oxidation of [^{14}C]-palmitate was also seen under the conditions of combined carnitine and sucrose administration (Fig. 6).

The present results show that *L*-carnitine counteracts the carbohydrate – induced hyperlipidaemia. A high intake of dietary sucrose increases the blood triglyceride and free fatty acid concentration. The hyperlipidemic effect of sucrose is associated with the intense conversion of fructose to fatty acids and triglycerides in the liver. The sucrose-rich alimented rat is a good model with respect to the endogenous carbohydrate-induced hypertriglyceridaemia. This model was used in order to test mechanisms of the influence of carnitine on blood lipid levels. In view of the question whether exogenous carnitine effects not only fatty acid oxidation but also other metabolic pathways, the activities of lipogenic liver enzymes were measured. According to our results, the activities of citrate cleavage enzymes, malic enzyme, glucose-6-phosphate dehydrogenase, and 6-phosphogluconate dehydrogenase are increased under the conditions of sucrose-rich diet. *L*-Carnitine has no effect on these enzyme activities and it can be concluded that carnitine administration does not change the lipogenic capacity of the liver, but an increased rate of fatty acid oxidation was found. The latter seems to be the primary mechanism of the counteraction of *L*-carnitine also under the conditions of sucrose-induced hyperlipidaemia.

Several studies suggested that carnitine may be useful in the treatment of hypertriglyceridaemia and muscle carnitine deficiency states induced during haemodialysis. Type IV hyperlipidaemia of patients on dialysis is reversed by carnitine therapy [10, 23]. According to *Ramacci et al.* [24] carnitine counteracts triglyceride and cholesterol accumulation in the liver, which is closely related to the sucrose-induced hypertriglyceridaemia of the rat. Furthermore, *L*-carnitine could reduce serum triglycerides only in hypertriglyceridemic patients [9]. Our investigations extend these observations with regard to the model of the sucrose-induced hyperlipidaemia and show that lipogenic liver enzymes are not influenced. However, the rate of fatty acid oxidation is increased. This is in accordance with clinical studies which demonstrated that oral *L*-carnitine supplementation results in an increase in long-chain fatty acid synthesis in subjects without carnitine deficiency [25].

The carnitine dose per body mass used in our animal experiments corresponds to the 2–3 fold used in clinical studies. According to our results, exogenous *L*-carnitine is able to regulate the metabolism of fatty acids by facilitating their intramitochondrial oxidation also under the conditions of carbohydrate-induced hyperlipidaemia. The most important result of our study is the triglyceride lowering effect of *L*-carnitine under the conditions of a sucrose-rich diet. We suggest the use of this physiological compound in the therapy of hypertriglyceridaemias.

Experimental

Female Wistar rats weighing *ca.* 300 g and fed a standard pellet diet were used. To test the effect of *L*-carnitine, the animals were divided into a control and 3 experimental groups. The control groups received standard food and drinking water *ad libitum*. The second group received standard food and *L*-carnitine in the drinking water (11 mg per day and 100 g body weight) over the period of 1 week. The third group was given standard food and a 10% sucrose in drinking-water, and the fourth group of

animals received sucrose combined with *L*-carnitine. The sucrose intake was around 30% of the total energy uptake and the daily energy intake was balanced between the different groups.

The rate of palmitate oxidation (fatty acid oxidation) in the liver was measured with [^{14}C]-palmitate [26]. Synthesis of fatty acid (palmitic acid equivalents) in the liver was determined with the Tritium water method [27]. The determination of lipogenic enzymes in the supernatant of liver homogenate was carried out with the optical test in the kinetic analyser (LKB Producter AB, Bromma Sweden): citrate cleavage enzyme (EC 4.1.3.8), malic enzyme (EC 1.1.1.38), glucose-6-phosphate dehydrogenase (EC 1.1.1.49), and 6-phosphogluconate dehydrogenase (EC 1.1.1.43). Serum triglycerides and free fatty acids were assayed by enzymatic kits (CHOD-PAP method, Boehringer Mannheim, Mannheim, Germany). The statistical analysis was carried out with the U-test by *Mann* and *Whitney*.

References

[1] Rebouche CJ, Paulson DJ (1986) Ann Rev Nutr **6**: 41
[2] Tomomura M, Imamura Y, Tomomora A, Toriuchi M, Saheki T (1994) Biochim Biophys Acta **18**: 307
[3] Pistorio E, D'Amico G, Pistorio A (1996) Clin Ter **147**: 403
[4] Seim H (1996) The Role of Catabolic Reactions and Nutrition for the Etiopathogenesis of Carnitine Deficiency Syndroms. In: Seim H, Löster H (eds) Carnitine – Pathobiochemical Basics and Clinical Applications. Ponte Press, Bochum, p 123
[5] Karlic H, Lohninger A, Laschan C, Lapin A, Bohmer F, Huemer M, Guthann E, Rappold E, Pfeilstocker M (2003) J Mol Med **81**: 435
[6] Pauly DF, Pepine CJ (2003) Am J Kidney Dis **41**: 35
[7] Steiber AL, Weatherspoon LJ, Spry L, Davis AT (2004) Clin Nutr **23**: 27
[8] Stefanutli C, Vivenzio A, Lucani G, Di Giacomo S, Lucani E (1998) Clin Ter **149**: 115
[9] Elisaf M, Bairakari E, Katopodis K, Pappas M, Steropoulos G, Tzallas C, Tsolas O, Siamopoulos KC (1998) Am J Nephrol **18**: 416
[10] Bouvier G (2002) HIV Clin **14**: 8
[11] Pola P, Savi L, Grilli M, Flore R, Serrichio M (1980) Curr Ther Res **27**: 208
[12] Richter V, Rassoul F, Würzberger G, Seim H (1996) Hyperlipoproteinaemias and Carnitine: Animal Experiments and Clinical Investigations. In: Seim H, Löster H (eds) Carnitine – Pathobiochemical Basics and Clinical Applications. Ponte Press, Bochum, p 195
[13] Ukropec J, Reseland JE, Gasperikova D, Demcakova E, Madsen L, Berge RK, Rustan AC, Klimes I, Drevon CA, Sebokova E (2003) Lipids **38**: 1023
[14] Maccari F, Arseni A, Chiodi P, Ramacci MT, Angelucci L, Hulsmann WC (1987) Lipids **22**: 1005
[15] Shimura S, Hasegawa T (1993) J Vet Med Sci **55**: 845
[16] Bhuiyan J, Seccombe DW (1996) Lipids **31**: 867
[17] Mondola P, Santillo M, De Mercato R, Santangelo F (1992) Int J Biochem **24**: 1047
[18] Sayed-Ahmed MM, Khattab MM, Gad MZ, Mostafa N (2001) Pharmacol Res **44**: 235
[19] Spagnoli LG, Orlandi A, Marino B, Maurello A, De Angelis C, Ramacci MT (1995) Atherosclerosis **114**: 29
[20] Löster H (2000) Ann Nutr Metab **44**: 82
[21] Vrana A, Fabry P (1983) Wld Rev Nutr Diet **42**: 56
[22] Okazaki Y, Katayama T (2003) J Nutr Biochem **14**: 81
[23] Glogger A, Bulla M, Furst P (1989) Kidney Int (Suppl) **27**: S256
[24] Ramacci MT, Maccari F, Zago E, Ghirardi O (1982) Effect of *L*-Carnitine on Lipid Metabolism: Preliminary Experimental Results. In: Ricci G (ed) Therapeutic Selectivity and Risk Benefit Assessment of Hypolipidemic Drugs. Raven Press, New York, p 273
[25] Mueller DM, Seim H, Kiess W, Löster H, Richter T (2002) Metabolism **51**: 1389
[26] Mc Carry JD, Takabayashi Y, Foster DW (1978) J Biol Chem **253**: 8294
[27] Goubern M, Portet R (1986) Ann Nutr Metab **30**: 380