Relationship between DNA and cholesterol synthesis in kidney after refeeding

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Summary. DNA and cholesterol synthesis were investigated in the kidneys of fasted-refed rats. Refeeding resulted in an increase in kidney DNA synthesis, as measured by ^{3}H-thymidine incorporation, starting at 72 h. The increase in DNA synthesis was accompanied by a stimulation of cholesterol synthesis, as measured by ^{14}C-acetate incorporation into cholesterol.
Key words. Rat kidney; cholesterol synthesis; DNA synthesis; refeeding; proliferative response; insulin.

Over the past few years, evidence has accumulated to suggest a positive correlation between cholesterogenesis and DNA synthesis. Cholesterol biosynthesis is a necessary prerequisite during cell proliferation, not only to produce the cholesterol needed for membrane biogenesis, but also to produce mevalonic acid, which has recently been shown in vitro to serve as a rapid and essential initiator in DNA replication [1]. A strong association between these metabolic pathways was also found in our laboratory in different models of hepatic cell proliferation, including liver of fasted-refed rats [2–4]. Normal adult liver is an organ which has a very slow cell turnover, but actively synthesizes cholesterol for export purposes. The question arises as to whether a concomitant increase of DNA and cholesterol synthesis could also occur in an organ, such as kidney, which has very low cell turnover and a very low capacity for synthesizing cholesterol [1], if it is stimulated to proliferate.

Since it has been shown that fasting-refeeding is able to cause cell proliferation not only in the liver but also in other organs such as intestine [5] and gall bladder [6], the first aim of the present study was to investigate whether refeeding is able to induce DNA synthesis also in the kidney. In addition, cholesterol synthesis was also studied, in order to verify whether the association between this metabolic pathway and DNA synthesis that had been observed in the liver, could also occur in the kidney following refeeding.

Materials and methods. Male Wistar rats, of the same age, weighing 200–250 g, were used in these experiments. Unless otherwise stated, they had free access to water and to a semisynthetic diet (Ditta Piccioni, Brescia, Italy). All experiments were performed with animals sacrificed at the same time during a controlled dark-light cycle.

The rats were fasted for 72 h and refed for 24, 48, 72, 96, 120 and 168 h prior to sacrifice. All animals were sacrificed by exsanguination under ether anesthesia. The kidneys were resected and immediately processed for analyses.

DNA and cholesterol synthesis were determined as described previously [7].

Results. As shown in the table, 72 h of fasting resulted in a reduction of kidney DNA and cholesterol synthesis (36% and 88%, respectively) when compared to the fed controls. Refeeding caused a prompt stimulation of these metabolic pathways, which were found to be significantly more active than in the 72-h fasted group after 24 h.

The level of cholesterol synthesis was significantly higher than that of the fed controls 48 h after refeeding, and the level of DNA synthesis was higher after 72 h.

DNA and cholesterol synthesis in kidney of fasted-refed rats

	Incorporation of ^{3}H-thymidine (cpm/μg DNA)	Incorporation of ^{14}C-acetate (cpm/mg cholesterol)
Fed controls (4)	8.64 ± 0.50**	2890 ± 241**
Fasted controls (4)	5.55 ± 0.66*	368 ± 22*
Refeeding (h)		
24 (4)	9.20 ± 0.69**	2286 ± 408**
48 (4)	8.73 ± 0.66**	5215 ± 833* **
72 (4)	21.31 ± 4.10* **	8702 ± 234* **
96 (4)	20.22 ± 2.51* **	6414 ± 559* **
120 (4)	25.68 ± 5.03* **	5968 ± 551* **
168 (4)	31.94 ± 6.79* **	5783 ± 781* **

The values are expressed as the mean ± SE. The number of animals is given in parentheses. Statistical significance of the difference, p-values by t-test. Differences between means were considered significant if p was less than 0.05. * $p < 0.05$ vs fed; ** $p < 0.05$ vs fasted.

Discussion. In the present study, the proliferative response of kidney cells to refeeding was investigated in fasted rats. An increased incorporation of ^{3}H-thymidine into kidney DNA was found after 72 h of refeeding. A similar effect was found in other organs, such as intestine [5] and gall bladder [6], and recently also in the liver [4]. The proliferative effect is probably mediated by the release of insulin from the pancreas, following the refeeding stimulus. Insulin is in fact well known to have growth-promoting action in various types of mammalian cells [8].

The rate of DNA synthesis was recently found to be positively correlated with cholesterol synthesis in different models involving hepatic cell proliferation [2–4]. The same correlation was found in this study in kidney, an organ that, in contrast to the liver, is virtually devoid of cholesterol synthesis under normal resting conditions. This strongly supports the concept that de novo cholesterol synthesis, and not simply cholesterol itself, is required during cell proliferation.

1 Siperstein, M. D., J. Lipid Res. *25* (1984) 1462.
2 Dessi, S., Batetta, B., Laconi, E., Ennas, C., and Pani, P., Chem. Biol. Interactions *48* (1984) 271.
3 Dessi, S., Chiodino, C., Batetta, B., Fadda, A. M., Anchisi, C., and Pani, P., Exp. molec. Path. *44* (1986) 169.
4 Dessi, S., Chiodino, C., Batetta, B., Armeni, M., Mulas, M. F., and Pani, P., Pathology *20* (1988) 53.
5 Stragand, J. J., and Hagemann, R. F., Experientia *34* (1978) 189.
6 Putz, P., Bazira, L., and Willems, G., Experientia *37* (1981) 744.
7 Ledda-Columbano, G.M., Columbano, A., Dessi, S., Coni, P., Chiodino, C., Faa, G., and Pani, P., Chem. biol. Interact. *62* (1987) 209.
8 Carpenter, G., Epidermal growth factor, in: Tissue Growth Factors. Hbk exp. pharmac. *57* (1981) 89.

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