Sex-dependence in triglyceride metabolism in response to dietary carbohydrates

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Summary. In humans, as well as in mice, fed on high carbohydrate diets, there was a significant sex difference in the plasma triglycerides in that males had higher levels than females. This was mainly due to the difference in their removal rate of circulating triglycerides in the animals of both sexes. In mice, males had higher levels of liver triglycerides as well as higher rate of incorporation of U-^{14}C-glucose into liver triglycerides when compared to females.

Elevated plasma triglycerides besides cholesterol have gained increasing importance as one of the risk factors of ischaemic heart disease (IHD)[2]. It is widely believed now that women of reproductive age are less prone to various cardiovascular diseases than men[3]. The death rate due to IHD in age group of 35–55 years is reported to be 6 times higher in males than females[3]. Although several studies have indicated that feeding of high carbohydrate diet leads to hypertriglyceridemia in humans[4,5] and in experimental animals[6,7], the sex dependence on these effects have not been adequately investigated. Macdonald[8] reported that high carbohydrate feeding resulted in elevated plasma triglyceride levels in men, while no such effect was seen in women of reproductive age. This observation was confirmed later by Jourdan[9], who reported a similar sex difference in plasma lipids in response to i.v. injected fructose (20%) in baboons. We report here increased plasma triglycerides and their decreased turnover in male subjects receiving carbohydrate-rich diet, in comparison with females. Similar sex difference was also observed in mice maintained on high sucrose diet with respect to plasma and liver triglycerides and in the fractional turnover rate of the plasma triglycerides.

Materials and methods. Eleven each of male and female healthy volunteers (staff and students of this institute), of an average age of 35 and 29 years, respectively, receiving carbohydrate-rich diet (70% in the form of starch and sucrose) were included in the study. Blood samples were taken from the subjects fasted overnight, into heparinized tubes and plasma was separated. The plasma was treated with isopropanol (1:10, v/v)[10] and the lipids were processed and triglycerides were estimated by the method of Van Handel and Zilversmit[11]. The i.v. fat tolerance test was performed with intralipid (Vitrum, Stockholm, Sweden) and the fractional turnover rate (K_2, min^{-1}) of the fat emulsion was estimated[12].

In the experiments on animals, mature male and female mice, weighing approximately 20–25 g each (3–4 months old), were adapted to a basal diet (containing sucrose, 50%; casein, 20%; butter fat, 20%; cellulose powder, 5%; salt mixture, 4%; vitamin mixture, 1%) for 2 weeks. These animals were then switched on to a fat-free high sucrose (70%) diet for 12 days. Our previous experiments showed that the peak of triglyceridemia levels was reached in 12 days by feeding mice on the high sucrose diet. The blood was removed by heart puncture under light ether anaesthesia in tubes containing heparin. The plasma was separated, lipids were extracted and the triglyceride estimated as described above. The secretion rate of plasma triglycerides from liver, at the time of peak triglyceridemia, was estimated at 8 h after an i.p. injection of triton WR 1339 (10 mg/mouse) to animals previously fasted overnight[13]. Removal rate (fractional turnover rate) of circulating triglycerides was estimated by injecting 0.1 ml of 10% intralipid per mouse and calculating the disappearance rate[14]. A separate batch of animals was injected i.p. with 2 μCi of U-^{14}C-glucose, and 2 h later the animals were sacrificed and liver removed. Liver lipids were extracted with chloroform-methanol, 2:1 (v/v) separated on thin-layer chromatography (TLC) and the triglyceride fraction was eluted from the silica gel[15]. This eluate was divided into two parts, one was used for estimating triglycerides[11] and the other was counted for radioactivity. The results were expressed as mean ±SEM and were statistically analyzed by Student's t-test.

Results and discussion. The data on humans revealed that plasma triglycerides were significantly lower in females when compared to males (table 1). The low plasma triglycerides in females was associated with higher removal rate

Table 1. Plasma triglyceride levels and intralipid clearance rates in 11 male and female adult Indians

Sex	Number of subjects	Average age, years	Plasma triglycerides (mg/100 ml)	Fractional turnover rate of intralipid (K_2, min^{-1})
Male	11	35	106.0±8.8	0.0319±0.0026
Female	11	29	82.0±8.0*	0.0467±0.0033**

* $p<0.05$; ** $p<0.01$.

Table 2. Some aspects of lipid metabolism of mice fed on high sucrose diet for 12 days

Experiment No.	Parameters	Male	Female	p-value
1	Fasting plasma triglycerides (mg/100 ml)	128.0±4.5 (8)*	108.2±4.6 (10)	<0.01
2	Secretion rate of plasma triglycerides:			
	a) Plasma triglycerides of saline treated animals** (mg/100 ml)	116.0±3.6 (6)	98.3±3.9 (5)	-
	b) Plasma triglycerides of triton treated animals**	246.5±10.5 (6)	211.7±6.8 (5)	-
	c) Increase over basal level (%)	112.4	115.4	ns
3	Fractional turnover rate of intralipid (K_2, min^{-1})	0.081±0.006 (7)	0.166±0.009 (6)	<0.001
4	Liver triglycerides (mg/g wet wt)	46.2±1.4 (6)	34.5±2.3 (6)	<0.01
5	Incorporation of U-^{14}C-glucose into liver triglycerides (d.p.m./mg triglyceride)	948±29 (6)	819±24 (6)	<0.01

* The number of animals in each experiment. ** The overnight fasted animals were injected with either 0.1 ml of saline or 0.1 ml of triton solution in saline (100 mg/ml), 8 h later blood was collected in heparinized tubes and plasma triglycerides estimated. ns: not significant.

(fractional turnover rate of intralipid) of circulating triglycerides in this group of population. This suggested an inverse correlation between plasma triglycerides and removal rate of this fraction.

The results on the metabolic studies of lipids in mice maintained on high sucrose diet are given in table 2. The triglycerides were found elevated both in males and females but the rise in males was significantly more than females. The possible reasons for higher levels in males could be either due to higher secretion rate from liver to plasma, or due to lower removal rate by extrahepatic tissues in comparison with females. However, there seemed to be no difference in the secretion rates of triglycerides as seen from the results of table 2. The fractional turnover of the triglycerides by extrahepatic tissue was significantly reduced in males when compared to that of females, indicating slower removal rate in males. Furthermore, there was higher rate of conversion of U-^{14}C-glucose into liver triglycerides as well as higher levels of triglycerides in liver of male mice than females.

Thus, the present studies in humans and in mice suggested higher plasma triglycerides in males, possibly due to slower removal rate. Macdonald[8] concluded in his study that the sex difference observed in plasma triglycerides in response to dietary carbohydrates could be due to the preventive effect of estrogen and progesterone in elevation of fasting serum glyceride. This was later disproved because administration of estrogens increased the serum triglyceride concentration, and this was associated with decrease in PHLA (postheparin lipolytic activity)[16,17]. The sex difference in triglyceride metabolism could, nevertheless, be due to effects of other hormones on the PHLA or clearance rates, the clarification of which needs a detailed investigation.

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Effect of exercise on ribonuclease activity in rat skeletal muscle

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Summary. Distribution of ribonuclease activity (measured at pH 7.6) in subcellular fractions of homogenates of rat skeletal muscle was investigated in sedentary animals and after 8 weeks running program. Training increased ribonuclease activity (expressed as units of enzyme per g of muscle protein). There was no increase in nuclear fraction, but in both cytoplasmic and mitochondrial fractions the RNA-ase activity increased 42% and 45% respectively.

It is commonly known that the adaptation of skeletal muscles to physical effort is closely connected with changes in metabolism, and to a high degree is the result of the regulation of the synthesis of biologically active proteins.

The results of several authors' studies make it possible to ascribe the regulation of protein biosynthesis at the stage of translation[2-5] and transcription[6-8] to ribonucleases. Until recently, there was no convincing proof that ribonucleases may fulfil the function of endonucleases described in degradation processes pre-m RNA to the form of cytoplasmic m-RNA.

The studies of Bardoń[9] and Libonati[10] would seem to confirm the role of intracellular alkaline ribonucleases in the processes of transformation of pre-m RNA into m-RNA. The biological role of RNA-ase, and our earlier results concerning changes in RNA-ase activity in blood serum following physical effort[11], led us to study the effect of physical exercise on the activity and distribution of alkaline ribonuclease in the skeletal muscles of rats.

Materials and methods. Training program. Investigations were carried out on male Wistar rats, weight 200–250 g. The animals were divided into 2 groups: a sedentary-control group, and a group subjected to running exercise on a motor-driven treadmill. All the animals were fed a standard diet.

The exercised animals ran at intervals for 8 weeks on a treadmill at an 8° incline. In the exercise cycle, the effort was increased from 4 2-min runs at a speed of 24 m/min, with 2-min intervals breaks, to 12 2-min runs at a speed of 48 m/min, with 2-min intervals breaks.

The joint power load each training day was increased from 4.5 to 27 W/kg and was kept at this level for the last 5 weeks of the experiment.