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Nagase analbuminemic rats have faster plasma triacylglycerol and VLDL synthesis rates

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Abstract

Serum triacylglycerol (TG) concentration is markedly elevated in Nagase analbuminemic rats (NAR) as compared to Sprague-Dawley rats (SDR) and reflects a high level of mainly VLDL. Hepatic production of triacylglycerol, as measured by the Triton-WR1339 infusion technique of impairing TG removal from blood, and plasma metabolic rate of pulse-infused [^{125}I]apo VLDL, were higher in NAR. However, contrary to previous reports, this elevated TG production could not be controlled by previous treatment of NAR with (i) bovine albumin infused intra-arterially or into the peritoneal cavity, or with (ii) dextran (Mol.wt. 73 500) injected intraperitoneally. Albumin administration expanded the plasma volume and could explain the apparent reduction of blood lipids found by others. Nonetheless, intraperitoneal dextran, as compared to saline, reduced the plasma cholesterol concentration regardless of the variation in the hematocrit level and thus, by raising the osmotic pressure of blood might regulate the metabolism of cholesterol-rich lipoproteins such as LDL and HDL in NAR.

Key words: Nagase analbuminemic rat; Hypertriglyceridemia; Hypercholesterolemia; Triacylglycerol synthesis; VLDL; Plasma lipoprotein composition; Albumin; Dextran

1. Introduction

Nagase analbuminemic rats (NAR) have extremely low plasma albumin concentrations and hyperlipidemia [1–3]. These animals are a useful model for the study of the interactions of plasma protein and lipoprotein metabolism. Experimental studies have not yet identified the origin of the defect leading to hyperlipidemia in analbuminemic, and hypoalbuminemic animals, such as nephrotic rats. Plasma albumin may regulate the metabolism of lipoproteins by several metabolic processes. Administration of albumin, which simultaneously corrects plasma oncotic pressure and other metabolic abnormalities, or of dextran, which only increases plasma oncotic pressure, reduces plasma lipid concentration in NAR [4] and in experimental nephrosis [5].

Reduced or absent plasma albumin levels may directly interfere with the transport of non-esterified fatty acids, or with the activity of the enzyme lipoprotein-lipase, or may influence the rates of hepatic synthesis of other proteins, including apoprotein constituents of lipoproteins, at the transcriptional-translational levels, or, indirectly, through mechanisms related to the regulation of blood oncotic pressure [6,5]. However, administration of high molecular weight polymers is unlikely to interfere with other metabolic processes, except the oncotic blood pressure [5]. On the other hand, it has been claimed that proteinuria itself, but not altered albumin metabolism, elicits hyperlipidemia in the nephrotic rat [7]. Therefore, metabolic alterations capable of interfering with plasma lipoproteins may be complex in NAR, as well as in the nephrotic syndrome. Accordingly, it has been claimed that NAR have low adipose tissue lipoprotein lipase activity [7,8] which should impair the removal of triacylglycerol-rich particles. Nonetheless, the plasma clearances of chylomicrons and of VLDL were apparently similar in NAR and Sprague-Dawley rats (SDR) [7]. Furthermore, high molecular weight polymers, as well as albumin administration, lower the blood lipid concentration

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without modifying the post-heparin lipase activity in neprotic rats [5]. Also, low liver lipid concentration in NAR may be compatible with a high rate of lipoprotein output from the liver, although experimental data were not presented to support this conclusion [9].

The objective of the present study was to identify the origin of the plasma lipoprotein abnormalities observed in NAR by determining the rate of plasma triacylglycerol input and whether this rate can be controlled by albumin or dextran infusion, and the rate of plasma metabolism of triacylglycerol-rich lipoproteins. These processes were quantified in NAR as compared to SDR.

2. Materials and methods

Nagase analbuminemic (NAR) and Sprague-Dawley male rats (SDR) were raised in conventional housing at 23°C. The animals were randomly chosen from the breeding colonies without previous knowledge of their plasma lipid levels and fed ad libitum a pelleted commercial chow diet provided by Nuvilab-Nuvital (S.P., Brazil). Animal body weights ranged from 250 g to 350 g but their weights were matched in all paired experiments.

Rat lipoprotein composition was analyzed in LP fractions from blood drawn by aortic puncture from animals in the fed state (between 11 a.m. and 3 p.m.). Blood was collected over heparin (20 I.U., 5 μl). LP were separated by discontinuos gradient ultracentrifugation [10] utilizing an SW41 rotor spinning at 200 000 $\times g$ (4°C) in an L8–70 M ultracentrifuge (Beckman Instruments, Palo Alto, CA). Chylomicrons were aspirated from the top $d < 1.006$ g/ml layer after a 30 min spinning period. Thereafter ultracentrifugation proceeded for 24 h and LP fractions were drawn at $d <$ 1.006 g/ml (VLDL), between 1.006 and 1.063 (LDL) and 1.063–1.210 g/ml (HDL). LP phospholipids were measured by the Bartlett method [11] and protein by the Lowry method [12]. Other lipids were analyzed by enzymatic methods: Cholesterol by Chod-Pap (Merck S.A., S.P., Brazil) and triacylglycerols by Enz-Color (Biodiagnostica, PR, Brazil).

All metabolic studies were carried out between 5 pm and 7 pm and 24 h after the pelleted diet had been discontinued but the animals had free access to both a 5% glucose solution and to plain water throughout the metabolic experiments. The animals were submitted to this long period of solid food deprivation in order to rule out the possible effects of plasma chylomicrons on the metabolic studies. After light ethyl-ether anesthesia, one carotid artery was cannulated with an indwelling polyethylene PE-50 catheter (Intramedic, Clay Adams, Parsippany, NJ) previously rinsed with liquid silicon (Silicone Prontosil, R.J., Brazil). The experiments were started 1 h later, with the animals restrained in Bollman-type cages.

Plasma albumin concentration was determined by agarose gel radial immunodiffusion using rat albumin standards and rabbit anti-rat albumin antibody (Organon Teknika, Durham, NC). In experiments where bovine albumin was infused intra-arterially, plasma bovine albumin was measured using bovine albumin standards and rabbit anti-bovine albumin antibody (Sigma, St. Louis, MO).

3. Experimental protocols

Plasma triacylglycerol (TG) synthesis rate was calculated as the increase in TG concentration over time after an intra-arterial pulse infusion of Triton-WR1339 (60 mg/100 g body wt.) which is known to interrupt the TG-rich lipoprotein clearance rate [13]: blood samples (100 μl) were drawn at 0, 30, 60 and 90 min. Time-dependent plasma TG secretion was obtained by a linear regression ($y = a + bx$) where b is the angular coefficient representing TG as mg/100 ml of plasma/min. In addition to comparing the SDR and the NAR groups, in two experiments it was also investigated whether raising plasma albumin in NAR might influence TG production. However, in these experiments plasma TG secretion rates were estimated using only the differences in plasma TG concentration between the values obtained at 90 min (after Triton-WR1339 infusion) and at 0 time. In one experiment this was accomplished by an intra-arterial pulse infusion of bovine albumin (400 mg/1.33 ml saline/rat) (Sigma, St. Louis, MO) during 30 s, immediately before the Triton-WR1339 infusion. NAR infused with saline alone (1.33 ml) prior to the Triton-WR1339 test were used as controls. All NAR were pre-selected for plasma TG concentrations within a narrow range and were randomly assigned to the two experimental groups. In addition, NAR blood hematocrit was measured prior to bovine albumin infusion, and blood hematocrit and bovine albumin levels in plasma were measured at the end of the experiment. In another study, bovine albumin (daily dose of 1 g/kg body wt) was administered intraperitoneally in 500 mg/ml saline for 3 days. A saline-treated group was utilized as control. On the fourth day the animals were submitted to the Triton-WR1339 test but plasma albumin concentration was not measured.

The influence of plasma oncotic pressure on NAR hyperlipidemia was investigated by the intraperitoneal infusion of Dextran (mol. wt. 73 500, Sigma, St. Louis, MO), 500 mg/1 ml saline per day, for 3 days; a saline-treated NAR group was used as control. On the fourth day blood was drawn from the dorsal tail artery prior to the measurements of plasma cholesterol, tri-

acylglycerol and hematocrit. Rats randomly used for study had been selected for plasma lipid values whithin narrow ranges: 180–260 mg/dl for cholesterol and 139 to 253 mg/dl for triacylglycerol.

Plasma clearance rate of [^{125}I]apo VLDL in NAR as compared to SDR was measured after intra-carotid pulse infusion of [^{125}I]VLDL. VLDL was obtained from a pool of SDR plasma donors submitted to sequential ultracentrifugation in a 50 Ti rotor [14]. After spinning at $d < 1.006$ g/ml at $105\,000 \times g$ (4°C) for 15 min the chylomicron-containing supernatant was discarded and VLDL was aspirated after a second ultracentrifugation lasting 15 h. VLDL was labeled with ^{125}I (N. England Nuclear, Boston, MA) by the Iodo-Gen procedure (Pierce, Rockford, IL). Radioactive VLDL was exhaustively dialyzed against EDTA-phosphate buffer (pH = 7.2) for 24 h, and an aliquot extracted with ethyl-ether: less than 3% of ^{125}I was present in the lipid component and 96–98% was precipitated by trichloroacetic acid.

Animals were in the fasting state as previously explained but given free access to water containing potassium iodide and iodine (1.5 mg each in 500 ml) also during the blood-drawing period. Blood samples (0.1 ml) were drawn sequentially over a period of 30 min and radioactivity was measured in 40 μl plasma aliquots using a gamma counter (Cobra model, Packard Instruments, Meriden, CT). Results expressed as fraction of the initial (30 s) radioactivity plasma value along time (30 min), represent the plasma residence time of [^{125}I]apo VLDL in minutes calculated as the area under the plasma radioactivity curve [15].

4. Results

Plasma triacylglycerol synthesis rates as measured by the Triton-WR1339 test are shown in Fig. 1. Production rate (mg/100 ml per min, mean ± S.D.) in NAR (1.93 ± 0.83, $n = 6$) was greater than in SDR (0.71 ± 0.57, $n = 5$) ($P < 0.05$). In another experiment in NAR ($n = 6$) that had been matched for having similar fasting triacylglycerol plasma concentrations, intra-arterial bovine albumin administration immediately before the Triton-WR1339 infusion test led to NAR albumin concentration rising from trace amounts to 1.7 ± 0.9 g/100 ml (mean ± S.D.) measured only at the end of the Triton-WR1339 experiment (at 90 min), yet the TG secretion rate was 0.85 ± 0.56 ($n = 3$) after albumin as compared to 0.80 ± 0.77 ($n = 3$) after saline, as measured by the 90 min. 0 min. differences in plasma TG values alone. Interestingly, there was a simultaneous decrement of the mean hematocrit value from 42.6% to 34.6% elicited by albumin administration as well as a variation from 42.7% to 37.2% in the saline-treated group. Also, chronic intraperitoneal albumin administration did not correct the faster TG synthesis rate in NAR, since the Triton-WR1339 test disclosed at 90 min a plasma TG input rate (mean ± S.D, mg.100 ml/min) of 2.34 ± 1.1 after albumin ($n = 5$) not differing from 2.09 ± 1.0 after saline ($n = 5$). Nonetheless, plasma albumin concentration was not measured in this experiment.

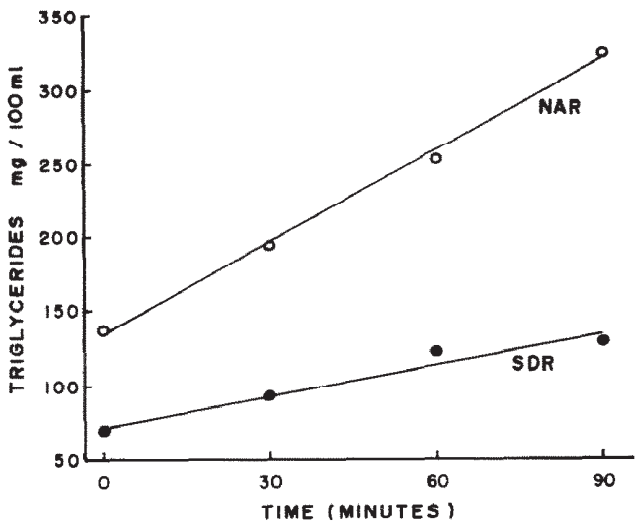

Fig. 1. Plasma triacylglycerol synthesis rates were measured in NAR ($n = 6$) and SDR ($n = 5$) after intra-arterial pulse infusion of Triton-WR1339 (60 mg/100 g body wt.). A time-dependent rise in plasma triacylglycerol (TG) was obtained by linear regression and represents TG mg/100 ml of plasma/min: 1.93 ± 0.83 in NAR and 0.71 ± 0.57 in SDR. Differences were significant at $P < 0.05$.

Intraperitoneal dextran and saline infusion had little effect on blood hematocrit (Table 1) and yet both treatments similarly reduced plasma triacylglycerol (TG) concentration in NAR. Thus, dextran infusion could not have influenced the metabolic rate of the triacylglycerol-rich particles. On the other hand, Dextran, but not saline infusion, significantly reduced the plasma concentration of cholesterol and thus must have influenced the metabolic rate of the TG-poor (cholesterol-richer) lipoproteins such as LDL and HDL.

Table 1
NAR plasma cholesterol and triacylglycerol (mean mg/100 ml ± S.D.) prior to and on the fourth day of intra-peritoneal infusion of dextran or saline (lasting 3 days)

	Cholesterol	triacylglycerol	hematocrit
Dextran			
Before	210 ± 22	166 ± 44	50 ± 4
After	172 ± 20	88 ± 17	49 ± 6
Saline			
Before	216 ± 22	173 ± 30	50 ± 3
After	220 ± 22	94 ± 9	56 ± 7

Data are presented before and after each treatment as mean mg/100 ml ± S.D. Statistical comparison by Student's 't' test:
Cholesterol: before × after dextran ($P < 0.01$); before × after saline (NS); triacylglycerol: before × after dextran ($P < 0.01$); before × after saline ($P < 0.001$).

The faster production rate of plasma TG in NAR shown by the Triton-WR1339 experiments could be attributed to the higher synthesis mainly of VLDL, which is rich in triacylglycerol. The metabolic rate of [^{125}I]apo VLDL particles from pooled donor SDR plasma did show a significantly shorter residence time in plasma (min. mean ± S.D.) when pulse infused into NAR (10.7 ± 2.7) as compared to SDR (15.2 ± 3.2) ($P < 0.05$) (Fig. 2).

The plasma lipoprotein composition of NAR as comparared to SDR is shown in Table 2. LP were obtained by discontinuous density gradient ultracentrifugation of plasma drawn during the fed phase. It is clearly seen that several lipid components of the lipoproteins were much higher in NAR than in SDR. Also, TG concentrations were consistently elevated in all lipoproteins in NAR. High chylomicron values in NAR may express a delayed particle removal rate due to competition for removal with VLDL. In regard to VLDL, four times higher TG levels and nine times higher apoprotein levels, but similar cholesterol and phospholipid levels in NAR as compared to SDR can be explained by a combination of much larger VLDL particle size and number in NAR. Plasma LDL of NAR contains almost three times as much TG and five times as much protein as SDR but, like in VLDL, similar cholesterol and phospholipids contents. Therefore, LDL particle number (according to protein levels) and size (according to triacylglycerols levels) seem much higher in NAR. In both groups, most of the plasma cholesterol content is present in high-density lipoproteins as has consistently been described in rats. Nonetheless, this fraction has a little less cholesterol and much more TG in NAR than in SDR.

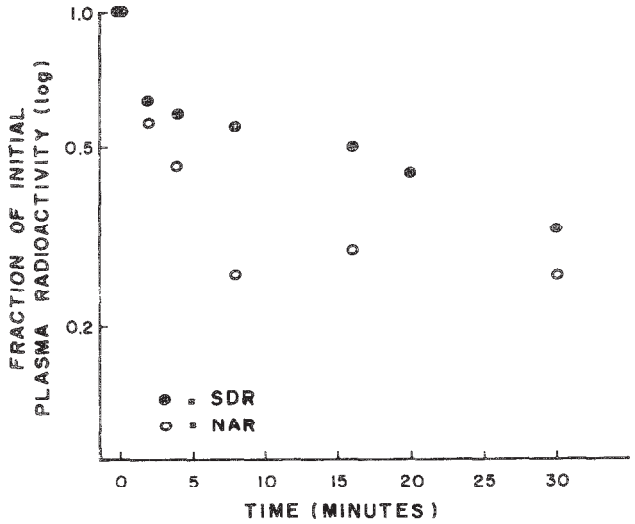

Fig. 2. Plasma clearance of pooled donor SDR [^{125}I]apo VLDL pulse infused into the carotid artery in NAR ($n = 5$) and in SDR ($n = 5$). Data expressing the fraction of the initial (at 30 s) radioactivity in plasma along time (30 min) were used to calculate the residence time of [^{125}I]apo VLDL as the area under the plasma radioactivity curves. Each point represents geometric means. Residence time (min. mean ± S.D.) was 10.7 ± 2.7 in NAR and 15.2 ± 3.2, in SDR, the difference being significantly different at $P < 0.05$.

Table 2
Plasma lipoprotein composition in NAR and SDR as measured in the fed state. Values as mean mg/100 ml ± S.D.

Chylomicrons [a]	NAR	SDR	Difference [b] p
Triacylglycerol	16.5 ± 4.5	3.7 ± 2.0	< 0.001
Cholesterol	2.0 ± 1.5	0.5 ± 0.3	< 0.05
VLDL			
Triacylglycerol	42.7 ± 12.9	11.5 ± 3.8	< 0.001
Cholesterol	7.5 ± 4.4	6.8 ± 2.1	NS
Phospholipid	1.7 ± 2.3	0.4 ± 0.2	NS
Protein	28.1 ± 19.3	3.2 ± 0.5	< 0.01
LDL			
Triacylglycerol	19.8 ± 6.3	7.3 ± 1.3	< 0.01
Cholesterol	29.2 ± 3.1	35.6 ± 12.8	NS
Phospholipid	1.6 ± 0.9	1.7 ± 0.8	NS
Protein	61.2 ± 5.3	12.0 ± 12.7	< 0.001
HDL [a]			
Triacylglycerol	29.8 ± 11.0	2.8 ± 0.5	< 0.001
Cholesterol	57.8 ± 9.0	68.4 ± 7.1	< 0.05
Phospholipid	5.1 ± 2.4	3.1 ± 1.5	NS

[a] Protein not measured.
[b] Comparison between NAR and SDR by Student's 't' test.

5. Discussion

Experiments in which the removal of TG-rich lipoproteins was suppressed by Triton-WR1339 (Fig. 1) clearly demonstrated a 2.7 higher TG secretion rate in NAR than in SDR. In this regard, the rate of [^{125}I]apo VLDL metabolism in plasma was 50% faster in NAR than in SDR which is a clear evidence that at least VLDL turnover is much faster in NAR. The apparent discrepancy between these two experimental results can be ascribed to the fact that triacylglycerol increase in NAR plasma is also due to other lipoprotein fractions (LDL and HDL) besides the VLDL component as shown in Table 2. However, Davies et al. [7] reported that the plasma clearance of VLDL in NAR was similar to SDR. Their VLDL was labeled in the triacylglycerol moiety with [^{3}H]glycerol, a procedure that may not identify the removal rate of the whole particle. In other words, since these authors showed a diminished lipoprotein lipase activity in NAR, which slows down the [^{3}H]glycerol-triacylglycerol disappearance rate but, when combined with a faster VLDL-synthesis rate hastens the disappearance rate of VLDL-apoprotein or core, the simultaneous recognition of both processes may have been impaired when

[^{3}H]glycerol was utilized. This mutual interference has been proposed by other authors in regard to the uptake of the chylomicron particle core [16]. In the present report, hepatic output of cholesterol was not investigated but the production of the cholesterol-rich LDL and HDL lipoproteins might simultaneously be enhanced in NAR since plasma LDL and HDL cholesterol concentrations were higher than in SDR (Table 2).

In the fed phase (Table 2) NAR have more TG in the chylomicron layer than SDR, which could be ascribed to competition with the high levels of VLDL for tissue removal. Thus, in a prolonged fasting state without solid food but with free access to water containing sugar, plasma TG must represent solely endogenous lipoproteins, particularly VLDL.

The present study utilizing the NAR model also aimed at shedding light on whether variations in plasma albumin concentration or in oncotic pressure interfere with the metabolism of plasma lipoproteins. Administration of albumin intra-arterially, or chronically through the peritoneal cavity, did not slow down the high triacylglycerol production rate in NAR as shown in the Triton-WR1339 experiment. Our results are at odds with those of other investigators who reported that albumin infusion reduced plasma lipid levels. In the experiment of Edelstein et al. [4] plasma albumin level obtained was 1.8 g/dl, a result similar to ours, and TG concentration at 4 h reduced 53%; in the experiments of Takahashi et al. [1] the same amount of albumin that was utilized in the present study (1 g/kg body weight) was injected only once into the peritoneal cavity raising the plasma albumin level while significantly reducing all plasma lipids, although transiently, since after three days all lipids rose to their original levels in plasma as the albumin concentration decreased. Proper controls for the albumin treated rats were not included. Although plasma albumin levels have not been measured in the present report, the three-day intraperitoneal albumin administration must have raised albumin concentration to normal values, as shown by others [1] and yet, as shown by the Triton-WR1339 experiment, (Table 1) failed to control the plasma TG input rate since similar results were obtained in the saline-treated group. A study on nephrotic rats [5] showed that similar treatments with albumin, gammaglobulin and polymers such as PVP, increase the plasma volume and decrease the lipid concentrations. However, none of these studies provided corrections of the lipid values by the expanded plasma volume disclosed by a fall in the hematocrit value. In the present investigation utilizing intra-arterial albumin, a 1.7 g/dl albumin level was reached, and blood hematocrit fell from 42.6% to 34.6%, corresponding to about 23% expansion of the plasma volume; the latter, nonetheless, cannot be attributed to albumin alone since a 15% volume expansion was also produced by the saline administration. Therefore, such treatments in rats bring about body fluid disturbances of unknown origin since they cannot be explained by the small volume (1.33 ml) administered, and yet increasing the plasma volume explains, to a considerable extent, the apparent reduction of plasma lipid concentration. After administering a large dose of Dextran intravenously, as done by Edelstein et al. [4], plasma volume could have expanded even to a greater extent than the value reached in present report. Nonetheless, in our study intraperitoneal Dextran and saline (Table 1) did not expand the plasma volume, and yet both procedures seemingly reduced the mean plasma TG concentration to the same extent, i.e., from 166 to 88 mg/dl and from 173 to 94 mg/dl, respectively. Thus, unknown metabolic factors secondary to animal handling diminish the quantity of plasma triacylglycerols independently of any specific effects related to the injection of the macromolecules themselves, such as albumin or polymers, like Dextran, and yet these findings had completely been ignored in previous studies that failed to carry out proper control experiments. On the other hand, in regard to the plasma cholesterol concentration, a plasma lipid-reducing effect was demonstrated in the NAR intraperitoneal Dextran experiment independently of the hematocrit which, incidentally, did not vary significantly (Table 1). Cholesterol is transported mostly by lipoproteins, like LDL and HDL, that contain much less TG than VLDL and chylomicrons. Taking into account that VLDL metabolism in NAR was not modified by the infusion of macromolecules, it is unlikely that less VLDL was converted to LDL or HDL as a consequence of these infusions. Probably macromolecules and polymers control the direct rates of production or uptake of cholesterol-rich lipoproteins that need further investigations in NAR, as well as in hypoalbuminemic nephrotic animals.

The present data identify a major metabolic abnormality in NAR, i.e., their higher TG production rate that can be ascribed to VLDL, although contributions from synthesis of LDL and of HDL, that are also rich in triacylglycerol, cannot be neglected. This metabolic alteration seems to be independent of control by the acute increase of the plasma albumin level, or by polymers that modify the plasma oncotic pressure. The latter, nonetheless, reduces the plasma cholesterol content by unknown mechanisms most likely interfering with the rates of metabolism of LDL and HDL, since these particles are cholesterol-rich. These findings in regard to VLDL metabolism support the conclusions drawn from studies on experimental nephrotic rats [7] in that neither hyperlipidemia nor defective lipoprotein metabolism are linked to albumin synthesis or serum albumin concentration, and, in the case of nephrosis, result at least in part, from proteinuria.

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