ELSEVIER

Atherosclerosis 158 (2001) 81–86

ATHEROSCLEROSIS

www.elsevier.com/locate/atherosclerosis

The rise of the plasma lipid concentration elicited by dietary sodium chloride restriction in Wistar rats is due to an impairment of the plasma triacylglycerol removal rate

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Received 28 February 2000; received in revised form 31 May 2000; accepted 20 December 2000

Abstract

Studies in humans have indicated that dietary salt restriction raises plasma levels of total cholesterol (TC) and triacylglycerols (TAG). In order to explain the mechanisms involved, a rat experimental model was developed consisting of chronic feeding ad libitum isocaloric diets with variable sodium chloride contents. Rates of synthesis of plasma TAG were measured either as the increase of plasma TAG after blocking its removal from plasma by the intra-arterial pulse infusion of Triton-WR 1339, or as the plasma rate of incorporation of [^{14}C]-oleic acid [^{14}C]-TAG. Plasma TAG removal rate was determined by the intra-arterial pulse infusion of a lipid emulsion. Severe salt restriction increased the plasma concentrations of TAG (71%) and of TC (10%). This result was not due to modification of the rate of synthesis of plasma TAG but was attributed to a 55% slower rate of removal of the TAG-containing lipoproteins. An increased plasma non-esterified fatty acid concentration, probably due to a salt restriction-related insulin resistance, may have impaired the activity of the enzyme lipoprotein lipase.

Keywords: Salt restriction; Triton-WR 1339; Triacylglycerol synthesis; Plasma lipoprotein kinetics; Hypertriglyceridemia; Hyperlipidemia

1. Introduction

Epidemiological studies have shown that arterial hypertension is often associated with dyslipidemia, although the mechanisms that characterize this association are not completely clear [1,2]. Reduction in dietary sodium chloride has usually been utilized to lower the arterial blood pressure of hypertensive patients and/or to protect normotensive subjects from the development of arterial hypertension [3]. However, a severe restriction of sodium chloride ingestion has shown adverse effects on glucose and lipid metabolism [4–7]. When submitted to a low sodium chloride intake, non-obese normotensive subjects present increased plasma triacylglycerol (TAG) and total cholesterol (TC) concentrations [6–9], and hypertensive, normotensive, as well as non-obese normotensive subjects develop increased fasting plasma insulin [4,6,7]. Furthermore, increased plasma TC and TAG concentrations were reported in hypertensive human subjects treated with the diuretic hydrochlorothiazide [10–12], and results regarding the effectiveness of this treatment on the prevention of coronary heart disease are controversial [13–16]. Hypercholesterolemia is a major risk factor for coronary artery disease (CAD) [17–20]. However, the elevated TAG and the low-antiatherogenic high-density lipoprotein cholesterol concentrations in plasma have also been associated with a high risk for CAD [21–28]. Therefore, the objective of the present study was to investigate in a rat model, the mechanisms involved in the variation of the plasma lipid concentration elicited by marked modifications in salt intake.

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0021-9150/01/$ - see front matter
PII: S0021-9150(01)00415-4

2. Materials and methods

2.1. Materials

[1-^{14}C]-Oleic acid was purchased from New England Nuclear (Boston, MA, USA); fatty acid-free albumin and Triton-WR 1339 were obtained from Sigma Chemical Co (St. Louis, MO, USA). Enzymatic kits for TAG and TC measurements were obtained respectively from Merck (Darmstadt, Germany) and Boehringer-Mannheim (Mannheim, Germany); enzymatic kit for non-esterified fatty acid (NEFA) measurement from Wako Chemicals (Richmond, VA, USA). Polyethylene PE-50 catheter was obtained from Intramedic Clay Adams (Parsippany, NJ, USA), and liquid silicone from Silicone Prontosil (RJ, Brazil).

2.2. Animals and laboratory methods

Newly weaned 3-week old male Wistar rats were fed ad libitum a pelleted chow diet provided by Harlam Teklad (Madison, WI, USA). The diet contained the following nutrients (g/100g): casein (28.7); sucrose (31.3); corn starch (20.0); soybean oil (6.0); minerals and vitamins. Added amounts of cellulose were substituted for sodium chloride so that its content in the diet was: low sodium diet, LSD (0.15); normal sodium diet, NSD (1.27); and high sodium diet, HSD (7.90). Animals were raised up to three months of age in conventional housing at 25°C on a 12:12 h light–dark cycle. Animals were randomly distributed into experimental groups differing according to the levels of sodium chloride intake. In order to avoid the presence of chylomicrons in plasma all metabolic studies were carried out in the morning period after a 12-h overnight fasting. Plasma TAG concentration (mg/dl, mean $\pm$ SD: 26 $\pm$ 8) measured after weaning and on a 12-h fasting period disclosed that the animal population was homogeneous. One week before the experiments blood (150 μl) was drawn over ethylenediaminetetraacetic acid (0.5%) for plasma NEFA measurements by enzymatic methods. Under light ethyl-ether anesthesia, carotid cannulation was performed utilizing an indwelling polyethylene PE-50 catheter, previously rinsed with liquid silicone. One hour after recovery from anesthesia, animals were restrained in a Bollman-type cage for the metabolic studies. Before the experiments, 150 μl of blood were drawn over heparin (20 IU, 5 μl) for TC and TAG measurements by enzymatic methods. Urinary sodium was measured with an FC280 flame spectrophotometer (CELM, SP, Brazil). Hematocrit was determined at the beginning and at the end of the all experiments.

2.3. Experimental protocols

In the first experimental protocol Wistar rats ate ad libitum a or a HSD. In a second set of experiments three groups of rats were fed ad libitum: LSD, NSD, and NSD with free access to a NaCl solution 0.9% (NSD + saline). Twenty four-hour urinary sodium ($U_{Na}V$) was measured as a control for sodium intake. In a third set of experiments two groups of animals (LSD and NSD) were fed ad libitum.

2.4. Triton-WR 1339 infusion test

Hepatic TAG synthesis rate was calculated as the rise over time in the plasma TAG concentration elicited by an intra-arterial pulse infusion of Triton-WR 1339 (60 mg/100 g body weight) which blocks the TAG-rich lipoprotein removal from plasma [29,30]. Plasma TAG and TC were analyzed in blood aliquots (100 μl) drawn at 0, 30, 60, 90 and 120 min after Triton-WR 1339 infusion, and the hepatic TAG synthesis rate was calculated by linear regression ($y = a + bx$), where b is the angular coefficient representing plasma TAG concentration/min [31].

2.5. [^{14}C]-Oleic acid infusion test

The hepatic TAG synthesis rate was measured utilizing an intra-arterial pulse infusion of a [^{14}C]-oleic acid–albumin complex (35 μCi/rat) [32]. [^{14}C]-Oleic acid is incorporated into hepatic TAG and delivered into the plasma compartment [33]. Thereafter, 120 μl of blood samples were drawn at 10, 20, 30, 40, 50, 60, 80 and 120 min for radioactivity measurement in plasma TAG which was separated by thin-layer chromatography [34]. The kinetic parameters of hepatic TAG synthesis were then determined by compartmental analysis of the plasma TAG specific radioactivity along time [33,35–37].

2.6. TAG clearance rate in vivo

After the initial blood sample drawing (100 μl), a 40% fat emulsion (Lipovenos®, Fresenius Laboratórios Ltda, SP, Brazil) was pulse infused intra-arterially (0.75 ml/kg body weight). The emulsion composition was as follows: 10% soya bean oil; 1.2% lecithin, and 2.5% glycerol. Plasma TAG was analyzed in blood aliquots (100 μl) drawn at 1, 3, 5, 7, 9, 12, 15 and 20 min after the fat infusion [38]. Plasma TAG clearance rate was calculated as the fractional catabolic rate (FCR), according to a compartmental analysis of the plasma TAG mass vs. time curves. The model is based on a two-compartment system, with an irreversible flow [39]. The compartmental analysis program em-

ployed is a modified SAAM (Simulation, Analysis And Modeling, Compartmental Analysis Program Manual, Institute of Nuclear Research (IPEN), University of São Paulo, São Paulo, Brazil, 1994).

2.7. Statistical analyzes

Data are expressed as mean ± S.D. Student's *t*-test was used for statistical evaluation of the data, except in the study that included three groups of rats (LSD, NSD and NSD + saline) where ANOVA with the Kruskal–Wallis post-test was used. Results of the statistical tests were considered to be significant at the 95% confidence level ($P < 0.05$).

3. Results

On ad libitum diet, LSD rats showed greater fasting plasma TAG and TC concentrations than HSD rats (Table 1). However, this finding could easily be explained by the fact that the latter group consumed less energy, reaching a lower final body weight. In order to circumvent a possible influence of body weight on plasma lipid levels, a second experimental protocol included LSD animals, NSD animals and also the NSD group receiving extra sodium chloride as a 0.9% NaCl drinking water solution. Accordingly, $U_{Na}V$ (mEq/24 h: mean ± SD) data for the LSD, NSD and NSD + saline groups were 0.22 ± 0.11 ($n = 8$), 1.65 ± 0.69 ($n = 6$), and 6.43 ± 1.72 ($n = 5$), respectively. Nonetheless, in this experimental protocol where all animals were also fed calories ad libitum and the NaCl intake varied markedly in the three experimental groups of rats, the LSD group remained hypertriglyceridemic and no differences in body weight or hematocrit values were observed among the three groups (Table 2).

In the third set of experiments, although LSD and NSD groups did not differ in body weight or hematocrit values, plasma NEFA and TAG concentrations were greater in the LSD animals (Table 3). Consequently, these results are explained solely by the use of low sodium, and not by differences in caloric intake.

The hepatic TAG synthesis rate (Table 4) did not differ between the LSD and the HSD groups as measured by the Triton-WR 1339 infusion or by the [^{14}C]-oleic acid–albumin complex infusion assay. However, in these experiments HSD rats attained lower body weight than the LSD rats. Nonetheless, in the separate set of experiments where a similar final body weight was attained by the LSD, NSD and NSD + saline animals, the Triton-WR 1339 infusion assay also showed that the hepatic TAG synthesis did not differ among groups (Table 5). On the other hand, the plasma TAG-FCR as measured after the intra-arterial lipid emulsion infusion assay showed lower values in the LSD than in the NSD group (Table 5).

Table 1
Characteristics of 12-week old Wistar rats fed ad libitum calories in a LSD as compared to a HSD during the previous nine weeks[a]

	LSD group	HSD group
TAG (mg/dl)	141 ± 67[b] ($n = 29$)	82 ± 39 ($n = 28$)
Cholesterol (mg/dl)	75 ± 10[b] ($n = 22$)	68 ± 12 ($n = 18$)
Body weight (g)	415 ± 46[b] ($n = 51$)	370 ± 45 ($n = 53$)
Hematocrit (%)	43.7 ± 5.3 ($n = 27$)	43.3 ± 5.6 ($n = 31$)

[a] Data are expressed as mean ± S.D.
[b] Statistical comparison by Student's *t*-test: LSD × HSD, $P < 0.05$.

Table 2
Characteristics of 12-week old Wistar rats previously fed for nine weeks ad libitum calories in diets containing LSD, NSD, or normal sodium and NaCl 0.9% (saline) added to the ad libitum drinking water (NSD + saline)[a]

	LSD group	NSD group	NSD + saline group
TAG (mg/dl)	107 ± 22[b,c] ($n = 10$)	80 ± 13 ($n = 10$)	77 ± 25 ($n = 9$)
Body weight (g)	377 ± 38 ($n = 10$)	350 ± 21 ($n = 10$)	350 ± 37 ($n = 10$)
Hematocrit (%)	49 ± 2 ($n = 10$)	50 ± 2 ($n = 10$)	46 ± 3 ($n = 9$)

[a] Data are expressed as mean ± S.D.
[b] Statistical comparison among all groups by one-way ANOVA: LSD × NSD, $P < 0.05$.
[c] Statistical comparison among all groups by one-way ANOVA: LSD × (NSD + saline), $P < 0.05$.

4. Discussion

In this study the low salt diet group was fed the minimum sodium chloride required for a normal rat growth rate [40]. However, the lower body weight attained by the HSD rats was ascribed to a diminished energy intake of a diet less agreeable to the animals [41] that at first glance might explain their lower plasma lipid levels. However, the introduction of the NSD and of the NSD + saline rat groups permitted us to obtain experimental protocols where there were no differences

Table 3
Characteristics of 12-week old Wistar rats previously fed for nine weeks ad libitum calories in diets containing LSD or NSD[a]

	LSD group ($n = 12$)	NSD group ($n = 10$)
TAG (mg/dl)	85 ± 3[b]	44 ± 3
NEFA (mEq/l)	1.45 ± 0.09[b]	1.06 ± 0.07
Body weight (g)	395 ± 15	364 ± 16
Hematocrit (%)	39 ± 2	40 ± 2

[a] Data are expressed as mean ± S.D.
[b] Statistical comparison by Student's *t*-test: LSD × NSD, $P < 0.05$.

Table 4
Hepatic TAG synthesis rate measured either as the rise in plasma TAG concentration/min after the Triton-WR 1339 intra-arterial pulse infusion or as the hepatic [^{14}C]-TAG synthesis rate estimated after [^{14}C]-oleic acid–albumin complex intra-arterial pulse infusion into the LSD and the HSD groups of rats[a]

	LSD group	HSD group
Triton-WR1339 (TAG mg/dl min)	14.0 ± 6.0 ($n = 11$)	13.0 ± 5.0 ($n = 10$)
[^{14}C]-TAG (min^{-1})	0.06 ± 0.03 ($n = 9$)	0.08 ± 0.04 ($n = 11$)

[a] Data are expressed as mean ± S.D. Statistical comparison by Student's t-test. Differences between experimental groups were not significant.

in animal body weight, and yet the low sodium intake also brought about a higher plasma TAG concentration. In the several groups of rats that had been submitted to the same diets, variations in plasma TAG levels can be ascribed to the fact that the animals employed in this study were non-isogenic Wistar rats and the experimental protocols were carried out in different occasions.

Interestingly, in the only experiment where plasma cholesterol and TAG were simultaneously measured (Table 1), the LSD group's TAG and TC were 71% and 10%, respectively, higher than in the HSD group. This finding in rats can be explained by the fact that, as opposed to humans, the largest share of plasma TC belongs to HDL [42–47], which seemingly is much less influenced by the diet salt content than the particles that are rich in TAG. In this regard, previous studies on humans had shown that a LSD brings on a simultaneous rise in plasma TC, which is represented mostly by the LDL fraction, and in plasma TAG, which belongs mostly to the VLDL fraction and chylomicrons [6–9]. In other words, variations in lipoprotein metabolism due to changes in salt intake levels may be more easily observed in humans than in rats.

The NSD + saline group had not been included in the design of the plasma TAG clearance study because this group and the NSD group did not differ in regard to the results obtained in the Triton-WR 1339 infusion test. In LSD, elevated plasma TAG occurred in the presence of normal TAG production as measured both by the Triton WR-1339 technique and by the [^{14}C]-oleic acid incorporation rate into plasma [^{14}C]-TAG. Nevertheless, the plasma TAG removal rate was significantly lower in the LSD than in the NSD group, as measured by the lipid emulsion intra-arterial pulse infusion. Since these metabolic studies had been carried out after a standardized fasting period where chylomicrons are no longer present in plasma [48,49], increased plasma TAG levels can only be ascribed to a plasma VLDL removal defect. In this regard, pilot LP preparative ultracentrifugation analyses carried out in fasting plasmas of rats that had been on LSD ($n = 2$) and on HSD ($n = 2$) diets disclosed that TAG concentration distribution in the VLDL and IDL fractions represented, respectively, 79.6% and 72.8% in the LSD, and 60.6% and 66.3% in the HSD rats. The present finding agrees with a previous report on obese mice, in which similar metabolic investigation procedures disclosed that hypertriglyceridemia is ascribed to impaired plasma TAG removal [50].

Variation in the metabolism of corticosterone, which is the most abundant adrenal corticosteroid hormone in the rat plasma [51], might be taken into account as an attempt to explain the higher plasma lipid level of the LSD group where an increase of the angiotensin blood level could have occurred and would then stimulate the production of aldosterone [52,53]. Corticosterone would increase since the desoxycorticosterone and corticosterone are direct precursors of aldosterone [54]. Furthermore, considering that corticosterone has glycocorticoid activity [55] this would then contribute to the rise of plasma NEFA and TAG levels consequently to a stimulated adipose tissue lipolysis rate [55,56]. However, this possibility was excluded because the blood corticosterone concentration (ng/ml; mean ± S.D) was not higher, and in fact was lower in the LSD as compared to the NSD group, respectively, being 266.4 ± 28.6, ($n = 17$) and 403.6 ± 25.5, ($n = 7$), $P < 0.05$. On the other hand, a lower plasma volume could have been brought about by a lower corticosterone concentration that, in turn, would produce falsely elevated plasma lipid levels. However, this possibility was ruled out because hematocrit values were not disturbed by any of the dietary salt contents.

Table 5
Hepatic TAG synthesis rates measured as the rise in plasma TAG concentration/min after Triton-WR 1339 intra-arterial pulse infusion into the LSD, NSD and NSD + saline groups and plasma TAG-FCR (min^{-1}) measured by lipid emulsion intra-arterial pulse infusion into LSD and NSD group of rats[a]

	LSD group	NSD group	NSD + saline group
Triton-WR1339[b] (TAG mg/dl min)	6.07 ± 1.56 ($n = 10$)	6.14 ± 1.17 ($n = 10$)	6.64 ± 1.14 ($n = 9$)
TAG-FCR (min^{-1})	0.052 ± 0.025[c] ($n = 11$)	0.117 ± 0.053 ($n = 9$)	

[a] Data are expressed as mean ± S.D.
[b] Differences among experimental groups were not significant by one-way ANOVA.
[c] Student's t-test: LSD × NSD, $P < 0.05$.

In a recent study utilizing the same animal model it was shown that LSD elicits a state of insulin resistance [57]. Furthermore, some investigations on hypertensive and normotensive humans submitted to a low salt diet have shown higher glycemia and insulinemia [4–6]. Thus, a low salt diet might bring about an insulin resistance state capable of lowering the activity of the enzyme lipoprotein lipase and enhancing the release of NEFA from TAG stores. Two consequences are expected from a greater plasma NEFA concentration which, incidentally, was found in the present study: (1) in the liver, NEFA is oxidized as well as incorporated into TAG and delivered into plasma as VLDL [27,58,59]. However, seemingly the latter was not the major effect of NEFA because a faster plasma TAG production rate did not occur; (2) impairment of the lipoprotein lipase activity [60–62] is the likely explanation because LSD rats, as compared to NSD rats, displayed the lower plasma TAG fractional metabolic rate.

Furthermore, our study sheds light on the mechanisms of the increased plasma TC and TAG concentration in hypertensive human subjects treated with the diuretic hydrochlorothiazide [10–12] and on the controversial results regarding the effectiveness of this treatment for the prevention of coronary heart disease [13–16].

Acknowledgements

The authors wish to acknowledge the contributions of Ubiratan F. Machado and Maristela M. Okamoto from the Institute of Biomedical Science of the University of São Paulo for corticosterone measurements; Rubens J. Silva in the preparation of the manuscript; Alice F.M. Shimabukuro, Elisabete A. Santos, Luzia N.S. Furukawa and Wagner V. Domingues for technical assistance; and Walter Campestre and Janice G.P. Silva for animal care. This work was made possible by the continuous and generous support from PRONEX (grant number 661092/1997-6), FAPESP, and Medical Laboratory Investigation of Hospital das Clínicas (LIM).

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