Journal of Cellular Biochemistry 63:349–357 (1996)

Nuclear Cholesterol Content and Nucleoside Triphosphatase Activity Are Altered in the JCR:LA-*cp* Corpulent Rat

Michael P. Czubryt, James C. Russell, John Sarantopoulos, and Grant N. Pierce

Ion Transport Laboratory, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre (M.P.C., J.S., G.N.P.) and the Dept. of Physiology, University of Manitoba, Winnipeg (M.P.C., J.S., G.N.P.), and Dept. of Surgery, University of Alberta, Edmonton (J.C.R.), Canada

Abstract A nuclear pore complex-associated nucleoside triphosphatase (NTPase) activity is believed to provide energy for nuclear export of poly(A)+ mRNA. This study was initiated to determine if nuclear membrane lipid composition is altered during chronic hyperlipidemia, and what effect this has on NTPase activity. The JCR:LA-*cp* corpulent rat model is characterized by severe hypertriglyceridemia and moderate hypercholesterolemia, and thus represents an ideal animal model in which to study nuclear cholesterol and NTPase activity. NTPase activity was markedly increased in purified hepatic nuclei from corpulent female JCR:LA-*cp* rats in comparison to lean control rats as a function of assay time, [GTP], [ATP], and [Mg^{2+}]. Nuclear membrane cholesterol and phospholipid content were significantly elevated in the corpulent animals. Nuclei of corpulent animals were less resistant to salt-induced lysis than nuclei of lean animals, suggesting a change in relative membrane integrity. Together, these results indicate that altered lipid metabolism in a genetic corpulent animal model can lead to changes in nuclear membrane lipid composition, which in turn may alter nuclear membrane NTPase activity and integrity.

Key words: hypercholesterolemia, nuclear membrane, NTPase, hyperlipidemia, obesity

The function of the nuclear nucleoside triphosphatase, or NTPase, is to provide energy to the nuclear pore complex for poly(A)+ mRNA export from the nucleus to the cytoplasm by the hydrolysis of nucleosides [Agutter et al., 1979; Clawson et al., 1980; Agutter and Prochnow, 1994]. The NTPase has been localized to the inner face of the inner nuclear membrane, placing it in an ideal position for its putative role, and has been demonstrated to be a protease cleavage product of lamins A and C, the primary constituents of the mammalian nuclear lamina [Clawson et al., 1984; Tokes and Clawson, 1989]. NTPase activity is stimulated by poly(A)+ mRNA [Schroder et al., 1986; Bernd et al., 1983]. Phosphorylation of the NTPase results in an increased affinity for mRNA and alterations in NTPase activity and translocation of mRNA out of the nucleus [McDonald and Agutter, 1980]. Together, these data and other work describe an enzyme able to fulfill the role of energy provider for pore complex mRNA export [Clawson et al., 1978; Agutter, 1980; Clawson et al., 1980; Schroder et al., 1984; Venkatraman and Clandinin, 1988; Schroder et al., 1988].

Despite the potential importance of this enzyme in gene expression, little is known about the capacity of the NTPase to adapt during a chronic disease state. Specifically, we were interested in the potential for the nuclear membrane to alter its cholesterol composition during disease and whether this change was associated with an alteration in nuclear NTPase activity. A relatively novel animal model, the JCR:LA-*cp* rat, carries the autosomal recessive *cp* (corpulent) gene first isolated by Koletsky [1973, 1975]. Rats that are homozygous for the *cp* gene (*cp*/*cp*) are obese, insulin resistant [Russell et al., 1987, 1994] and hyperlipidemic [Dolphin et al., 1987]. The hyperlipidemia is due to a marked hepatic hypersecretion of very low density lipoprotein (VLDL), leading to extreme hypertriglyceridemia and moderate hypercholesterolemia [Russell et al., 1989]. Animals that are homozygous normal (+/+) or heterozygous (+/*cp*) are

Received and accepted April 4, 1996.

Address reprint requests to Grant N. Pierce, Institute of Cardiovascular Sciences, St. Boniface General Hospital Research Centre, 351 Tache Avenue, Winnipeg, Manitoba, Canada R2H 2A6.

lean, with normal insulin and lipid metabolism. These animals offer a valuable model in which to examine the potential for membrane lipid content in hepatic nuclei to change in vivo in response to the high circulating lipid levels and to test if this change will alter NTPase activity. Our results provide the first evidence that (1) nuclear membrane cholesterol content can be altered in response to the environment, and (2) NTPase activity was significantly increased during a chronic disease state. Since NTPase activity may directly affect mRNA translation rates by controlling passage of mRNA to the cytoplasm, any factors which alter NTPase activity could have profound influences on cellular metabolism.

METHODS

Animals and Materials

Female JCR:LA-*cp* rats were bred in the established breeding colony at the University of Alberta using standard breeding protocol [Russell et al., 1989]. Lean rats from breeding +/*cp* animals are a mixture of +/+ and +/*cp* (1:2) and are designated +/?. Animals were given water and standard rat chow ad libitum until sacrifice by decapitation. Measurements of body, liver, and heart weight were recorded at time of sacrifice. Chemicals were purchased from Sigma Chemical Co. (St. Louis, MO) unless otherwise noted.

Isolation of Nuclei

Nuclei were isolated from freshly excised lean (+/?) or corpulent (cp/cp) female JCR:LA-*cp* rat livers according to previously reported methods [Czubryt et al., 1996a]. Purity was verified visually under the microscope using the DNA-specific dye Toluidine blue, and by assays for subcellular membrane marker enzymes which measured contamination by endoplasmic reticulum (mannose 6-phosphatase; [Gilchrist and Pierce, 1993]), plasmalemma membrane (K^+ *p*-nitrophenolphosphatase, Na^+-K^+ ATPase; [Pierce et al., 1989b]) and mitochondria (succinic dehydrogenase; [Pierce et al., 1989a]).

Protein Assay

Protein content of samples was determined with Markwell's variation of the method of Lowry to prevent interference from membrane lipids [Markwell et al., 1981].

NTPase Assay

Briefly, assays were completed in plastic tubes in 350 μl reaction buffer (250 mM sucrose, 20 mM MOPS, pH 7.4) containing EDTA (1 mM), $MgCl_2$ (1 mM or as required), and GTP or ATP (5 mM or as required). The reaction was started by the addition of 50 μl isolated nuclei (protein concentration ~3–10 $mg.ml^{-1}$) and carried out for 20 min (or as required) at 37°C. The reaction was stopped by the addition of 200 μl 10% sodium dodecyl sulfate (SDS). Inorganic phosphate generated in the reaction was measured by the method of Raess and Vincenzi [1980]. Identical assays were run as blanks, in which the SDS had been added prior to the reaction. NTPase dependence on time, [GTP], [ATP], and $[Mg^{2+}]$ was determined for both lean and corpulent animals, and Hanes K_M and V_{max} values determined. Free concentrations of ligands were determined from total concentrations using the Maxchelator program (© C. Patton, 1994).

Nuclear Membrane Integrity Assay

This assay was carried out as described previously [Czubryt et al., 1996b]. One milligram lean or corpulent rat liver nuclei was treated with STM buffer containing varying [NaCl] (0, 25, 50, 75, 100, 125, 150, 175, 200, 250, 300, or 1,000 mM) for 30 min on ice, then centrifuged at 7,500*g* in a microfuge. Supernatants were removed and nuclear nucleotide release into the supernatant estimated by absorbance readings in a spectrophotometer at 260 nm, with non-protein containing salt solutions as blanks. Data were normalized to the maximal absorbance values observed.

Nuclear Cholesterol and Phospholipid Content

Cholesterol and phospholipids were extracted from 100 μl isolated nuclei (0.3–1.0 mg total protein content) by first diluting with 600 μl water, then by adding 3 ml 2:1 chloroform: methanol and vortexing for 15 s. One milliliter water was added and the tubes vortexed for 15 s; then, 1 ml chloroform was added and the tubes again vortexed for 15 s. Samples were left to extract in the dark at 4°C overnight. The samples were then centrifuged in a table-top centrifuge for 10 min at low speed (750*g*). Following centrifugation, the upper, non-aqueous layer was removed by Pasteur pipette to a separate test tube and the solvent evaporated off under a stream of nitrogen gas. The dry samples were

resuspended in ethanol and assayed for cholesterol [Omodeo Sale et al., 1984] and phospholipid content [Kutryk and Pierce, 1988].

Serum Cholesterol, Glucose, and Triglycerides

Blood samples from lean and corpulent rats were centrifuged at low speed (750*g*) for 10 min and serum levels of cholesterol, glucose, and triglycerides were spectrophotometrically determined using commercial kits (Sigma; Stanbio Laboratory Inc., San Antonio, TX).

Statistical Analysis

Variation between sample means was determined with a two-tailed Student's *t*-test, with results $P < 0.05$ considered statistically significant. K_M and V_{max} values were calculated with the Hyper program (© J. S. Easterby, 1992), using Hanes plots.

RESULTS

Body and organ weights were recorded at the time of sacrifice of the animals (Table I). Both the body weight and the liver weight of the corpulent animals were significantly elevated compared to lean controls. However, both heart:body weight ratios and liver:body weight ratios were significantly decreased in the corpulent animals, likely due to the gross obesity of these animals.

Serum levels of glucose, cholesterol, and triglycerides were determined in both the lean and corpulent animals and found to be qualitatively similar to results reported previously (Table II) [Dolphin et al., 1990]. Glucose, cholesterol, and triglycerides were significantly elevated in the corpulent animals vs. the lean animals.

Nuclei purified from lean and corpulent livers were examined under the light microscope after

TABLE I. Body and Organ Weights of Female Lean and Corpulent Rats†

Animal	Body weight (g)	Heart weight (g)	Liver weight (g)	Heart:body ratio ($\times 10^3$)	Liver:body ratio ($\times 10^3$)
Lean	271 ± 16	0.97 ± 0.06	8.1 ± 0.3	3.6 ± 0.1	30.4 ± 0.7
Corpulent	502 ± 8*	1.04 ± 0.09	14.0 ± 0.4*	2.1 ± 0.1*	28.0 ± 0.7*

†Values represent means ± S.E. n = 10–22.
*$P < 0.05$ vs. lean animals.

TABLE II. Serum Levels of Glucose, Cholesterol, and Triglycerides†

Animal	Glucose (mg/dl)	Free cholesterol (μmol/ml)	Triglycerides (mg/dl)
Lean	131 ± 17	0.78 ± 0.11	43 ± 6
Corpulent	326 ± 18*	1.25 ± 0.17*	494 ± 42*

†Values represent means ± S.E. n = 4–6.
*$P < 0.05$ vs. lean animals.

TABLE III. Comparison of Marker Enzymes for Various Membranes*

Enzyme activity	Lean nuclei	Corpulent nuclei
Succinic dehydrogenase (pmol/mg/min)	16 ± 8	34 ± 4
Mannose 6-phosphatase (nmol/mg/min)	39 ± 8	40 ± 6
K^+-pNPPase (nmol/mg/hr)	34 ± 11	13 ± 3
Na^+-K^+ ATPase (nmol/mg/hr)	220 ± 170	130 ± 40

*Values represent means ± S.E. n = 3. $P > 0.05$ for lean nuclei vs. corpulent nuclei.

TABLE IV. Nuclear Content of Cholesterol and Phospholipids†

Animal	Cholesterol (nmol/mg protein)	% of lean	Phospholipids (nmol/mg protein)	% of lean
Lean	1.29 ± 0.49	—	13.6 ± 2.4	—
Corpulent	3.13 ± 0.32*	243	20.8 ± 1.8*	153

†Values represent means ± S.E. n = 3–7.
*$P < 0.05$ vs. lean animals.

TABLE V. Kinetic Parameters of the Nuclear NTPase†

	f(ATP)		f(GTP)	
Animal	K_M (μM)	V_{max} (nmol/mg/20 min)	K_M (μM)	V_{max} (nmol/mg/20 min)
Lean	89 ± 19	221 ± 8	155 ± 51	222 ± 32
Corpulent	200 ± 18*	342 ± 13*	283 ± 41	355 ± 27*

†Values represent means ± S.E. n = 3–5.
*$P < 0.05$ vs. lean animals.

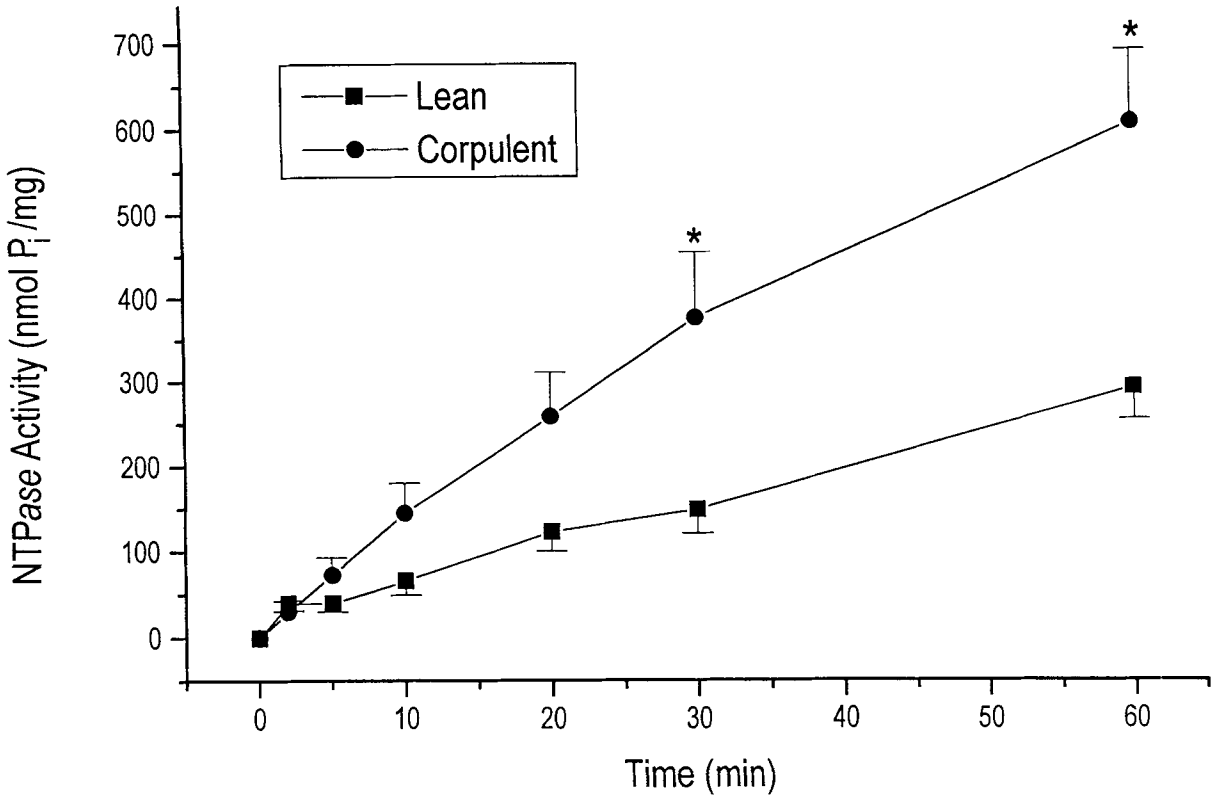

Fig. 1. NTPase activity in lean and corpulent 6-month-old JCR:LA-*cp* rat liver nuclei as a function of time. NTPase activity was assayed as described in the text, with [GTP] = 5 mM, $[Mg^{2+}]_{free}$ = 1 mM and [EDTA] = 1 mM at 37°C. Error bars represent standard error of the mean for 3–5 assays. *$P < 0.05$ vs. lean animals.

staining with the DNA-specific dye Toluidine blue. Visual examination revealed the nuclei to be round in shape with punctate nucleoli, hallmarks of liver nuclear morphology, with no visible contamination from cellular or other debris. No visible differences were observed between +/? and *cp/cp* nuclei.

A variety of marker activities for various subcellular membranes were examined to determine nuclear purity. There were no significant differences between purified +/? and *cp/cp* nuclei in any of the marker enzymes examined (Table III). Values measured are similar to those reported previously for highly purified nuclei [Czubryt et al., 1996a].

The cholesterol and phospholipid content of nuclei isolated from the corpulent and lean rats was measured. The nuclear cholesterol and phospholipid contents were significantly elevated in the corpulent animals compared to the lean animals (Table IV).

NTPase activity was examined as a function of reaction time. The liberation of P_i was initially linear. The rate then began to decrease after 20 min. NTPase activity was significantly elevated in the *cp/cp* animals compared to the +/? animals (Fig. 1).

NTPase activity was assayed as a function of [GTP] (Fig. 2). The *cp/cp* nuclei had significantly greater activity than the +/? nuclei. This resulted in a significantly higher V_{max} value observed for the *cp/cp* nuclei compared to the +/? nuclei (Table V). The plot of NTPase activity exhibited classic saturation kinetics for both types of nuclei. There was no significant change in the K_M affinity constant for GTP between *cp/cp* and +/? nuclei.

NTPase activity was also studied as a function of [ATP]. NTPase activity was significantly higher in *cp/cp* vs. +/? nuclei (Fig. 3), with the *cp/cp* nuclei also having a higher V_{max} value than the +/? nuclei (Table V). Classic saturation kinetics were also observed for both types of nuclei. The K_M affinity constant for ATP was higher in the *cp/cp* nuclei compared to the +/? nuclei.

Free magnesium is a required cofactor for NTPase activity. A logarithmic plot of NTPase

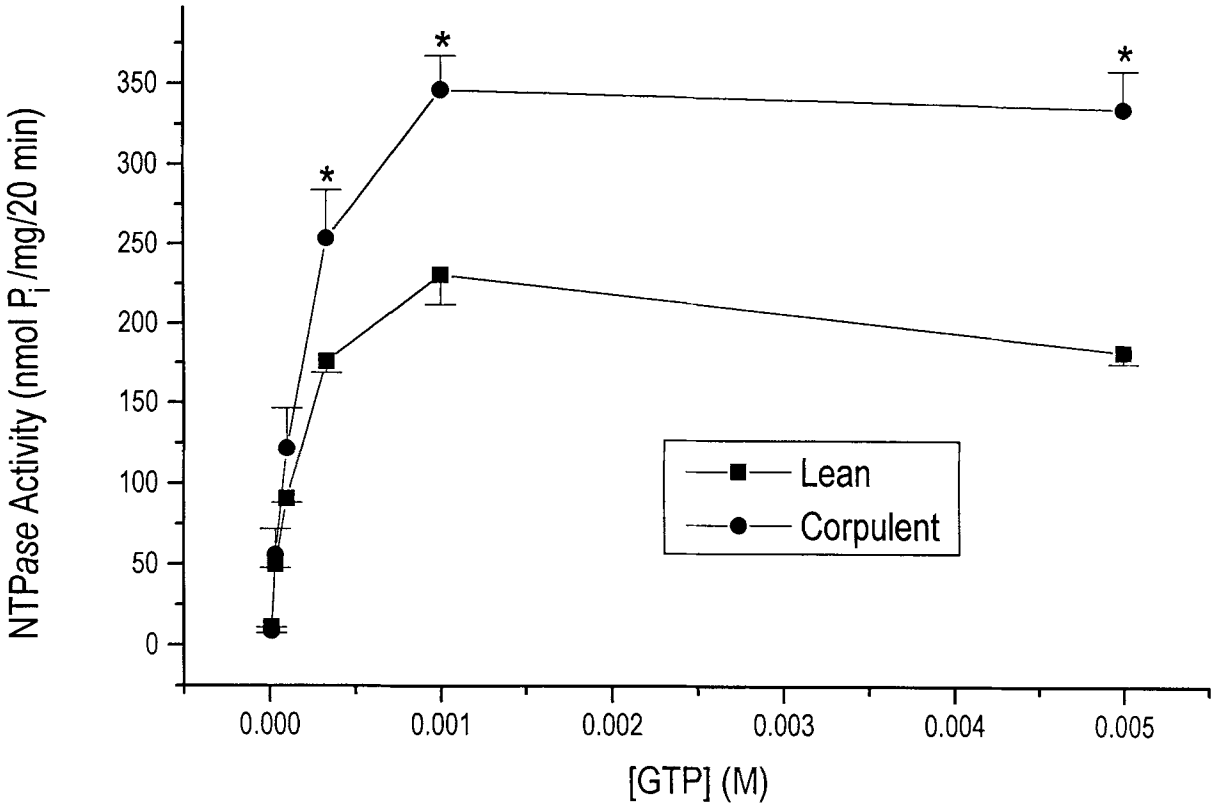

Fig. 2. NTPase activity in lean and corpulent JCR:LA-*cp* rat liver nuclei as a function of [GTP]. NTPase activity was assayed as described in the text, with $[Mg^{2+}]_{free}$ = 1 mM, [EDTA] = 1 mM and 20 min incubation at 37°C. Error bars represent standard error of the mean for 3–5 assays. *$P < 0.05$ vs. lean animals.

activity as function of $[Mg^{2+}]_{free}$ showed that the activity in *cp/cp* nuclei was significantly higher than in +/? nuclei only at $[Mg^{2+}]_{free}$ = 100 μM (Fig. 4). In both *cp/cp* and +/? nuclei, the shape of the plot was sigmoidal.

Assays were performed to investigate nuclear integrity during exposure to salt. An increased release of nuclear contents was observed at nearly every salt concentration tested in the corpulent animals compared to the lean animals (Fig. 5). Release of 50% of the nuclear contents (RC_{50}) occurred at a [NaCl] of 135 ± 7 mM in the corpulent animals, and at a [NaCl] of 158 ± 4 mM in the lean animals ($P < 0.05$).

DISCUSSION

Light microscopic examination of Toluidine-blue stained nuclei isolated from *cp/cp* and +/? rat livers revealed them to be normal in appearance (results not shown). Marker enzyme activities for contamination from other membranes revealed two features: first, there was no significant difference between +/? and *cp/cp* nuclei in any of the assays measured. Therefore, the isolation technique used provides equal levels of purity for both types of nuclei. Second, marker assay values obtained for purified nuclei are in most cases several orders of magnitude smaller than those obtained for purified subcellular membrane fractions, indicating the lack of contamination of the nuclei by other organelles [Czubryt et al., 1996a].

NTPase activity was significantly increased in the *cp/cp* nuclei compared to +/? nuclei as a function of time, [GTP], [ATP], and $[Mg^{2+}]_{free}$. V_{max} was about 50% greater for the *cp/cp* nuclei vs. the +/? nuclei for both GTP and ATP. The NTPase appears to hydrolyze GTP and ATP at equal rates as shown by the approximately equal (ATP vs. GTP) V_{max} values reported for both the +/? and the *cp/cp* rats. This is in agreement with previous reports [Schroder et al., 1986]. The NTPase activity curves for both GTP and ATP show classic saturation kinetics, i.e., smooth curves leveling off to a plateau value (V_{max}). These curves preclude the possibility that we may be measuring more than one hydrolytic enzyme. ATP exhibited a significantly elevated

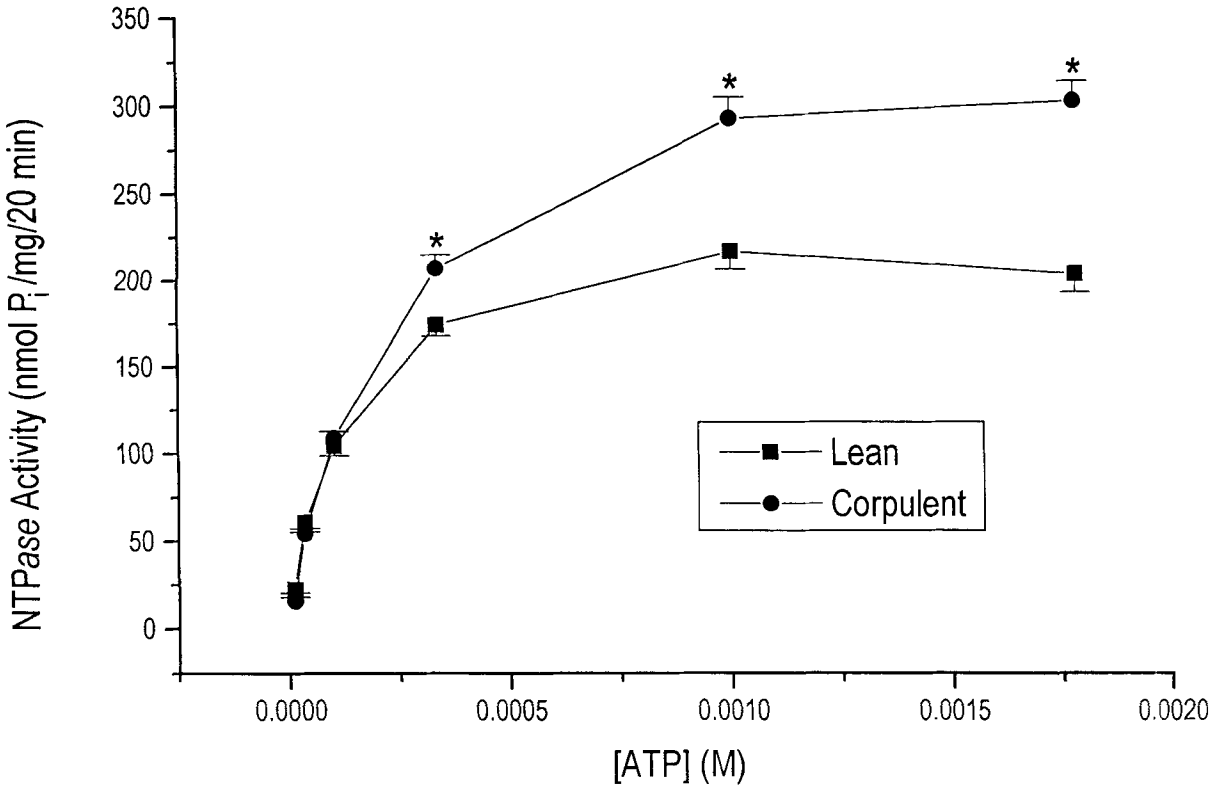

Fig. 3. NTPase activity in lean and corpulent JCR:LA-*cp* rat liver nuclei as a function of [ATP]. NTPase activity was assayed as described in the text, with $[Mg^{2+}]_{free}$ = 1 mM, [EDTA] = 1 mM and 20 min incubation at 37°C. Error bars represent standard error of the mean for 3–5 assays. *$P < 0.05$ vs. lean animals.

K_M value for the *cp/cp* nuclei vs. the +/? nuclei. This finding suggests that the affinity of the NTPase for ATP is reduced in the *cp/cp* animals, despite the increased V_{max} value also in the *cp/cp* animals.

The altered lipid composition of the nuclear membrane may be responsible for the alteration in NTPase activity. There were significant differences between +/? and *cp/cp* liver nuclei in both cholesterol and phospholipid content. The elevated levels of phospholipids in the *cp/cp* nuclei (153% of +/?) may contribute to the altered NTPase activity observed in this study. However, Smith and Wells [1984] observed that up to 80% of the phospholipids could be removed from nuclear membranes without altering the NTPase activity. When the nuclear membrane was completely delipidated, NTPase activity was abolished. From these findings, it would appear that although lipids are required for NTPase activity, the relatively small changes in phospholipid content observed here probably do not modulate this activity.

A more likely mechanism for the increased NTPase activity in the *cp/cp* animals, however, is the large increase observed in the nuclear membrane cholesterol content (243% of +/?). Increased cholesterol content has been shown to alter lipid membrane rigidity, which in turn may alter the activities of embedded enzymes [Shinitzky, 1984]. Many membrane-embedded enzymes and transporters have been shown to exhibit altered activity after manipulation of membrane cholesterol content [Kutryk and Pierce, 1988; Liu and Pierce, 1994; Shinitzky, 1984]. However, most importantly, when the nuclear membrane cholesterol content was increased by incubation of isolated rat liver nuclei with cholesterol-enriched liposomes, the NTPase was markedly stimulated [Ramjiawan et al., 1996]. The *percent* enrichment of cholesterol achieved in these studies (~275% of control) is comparable to that reported in the current work (243% of +/?). These data suggest that cholesterol is most likely responsible for the stimulation of NTPase observed in the current study.

The increase in nuclear membrane cholesterol may have implications other than just its effects on NTPase activity. In this study, nuclear membrane integrity was examined by measur-

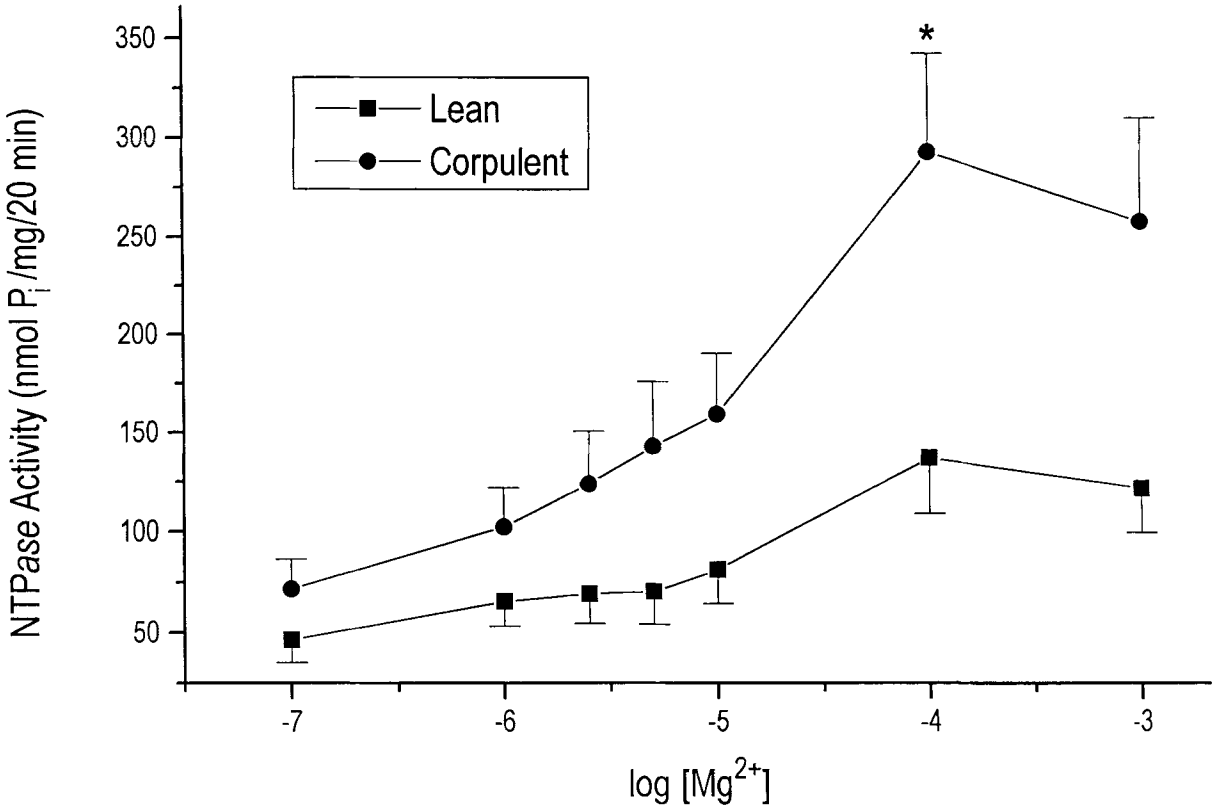

Fig. 4. NTPase activity in lean and corpulent JCR:LA-*cp* rat liver nuclei as a function of $[Mg^{2+}]_{free}$. NTPase activity was assayed as described in the text, with [GTP] = 5 mM, [EDTA] = 1 mM and 20 min incubation at 37°C. Error bars represent standard error of the mean for 3–5 assays. *$P < 0.05$ vs. lean animals.

ing the resistance of intact nuclei to salt-induced lysis as described previously [Czubryt et al., 1996b]. Nuclei of *cp/cp* rats released significantly more DNA at lower [NaCl] compared to nuclei of +/? rats. These findings suggest that the integrity of the *cp/cp* nuclear membranes was compromised compared to the +/? nuclear membranes. It is possible that the alterations in cholesterol content described above destabilized the nuclear membrane in the *cp/cp* nuclei, since cholesterol incorporation into isolated rat liver nuclei in vitro also compromises membrane integrity [Czubryt et al., 1996b; Ramjiawan et al., 1996].

This study examined NTPase activity in the JCR:LA-*cp* corpulent rat model. Although mRNA export from the nucleus was not studied directly, there is a large body of evidence showing that mRNA export and NTPase activity are closely linked. For example, blocking NTPase activity also inhibits mRNA export [Prochnow et al., 1994; Sidransky et al., 1984; Agutter et al., 1976; Schroder et al., 1984]. Conversely, increasing NTPase activity by various methods results in a stimulation of mRNA export [Sidransky et al., 1984; Schroder et al., 1990; Agutter et al., 1979]. It would, therefore, not be unreasonable to assume that the alterations reported here for NTPase activity may also reflect alterations in mRNA export. This may induce changes in gene expression, and potentially represents a novel mechanism for pathological changes in diseased cells.

In summary, this study has demonstrated for the first time that a corpulent rat model with abnormal lipid metabolism, the JCR:LA-*cp* rat, also has increased nuclear NTPase activity. This increase is likely due to significant increases in nuclear membrane cholesterol content. This alteration in nuclear membrane composition may also contribute to a change in nuclear membrane integrity. These findings have implications for nuclear function in other situations where abnormal lipid metabolism may alter the composition of cellular membranes, such as atherosclerosis [Chen et al., 1995; Kankaraj and Singh, 1989; Kummerow et al., 1994] or diabe-

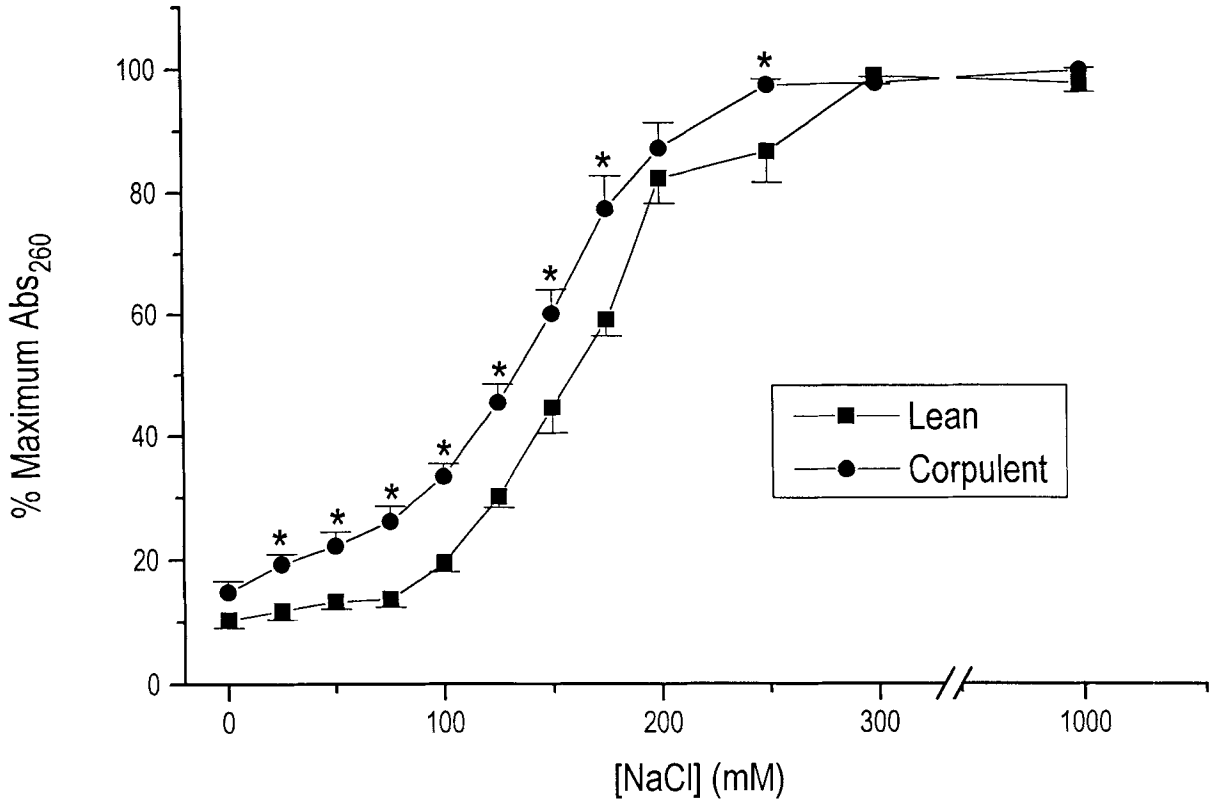

Fig. 5. Assay of membrane integrity in nuclei from lean and corpulent JCR:LA-*cp* rats. Aliquots of nuclei isolated from lean or corpulent rat livers were exposed to osmotic shock in the form of increasing [NaCl] and then measured for DNA release, as described in the text. Error bars represent standard error of the mean for 4–5 assays. *$P < 0.05$ vs. lean animals.

tes mellitus [Pierce and Dhalla, 1983; Pierce et al., 1983; Pelikanova et al., 1991].

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council of Canada. The authors thank Shoba Thomas for excellent technical assistance. G.N.P. was a Scientist of the Medical Research Council of Canada. M.P.C. was supported by a graduate student fellowship from the University of Manitoba.

REFERENCES

Agutter PS (1980): Influence of nucleotides, cations and nucleoside triphosphatase inhibitors on the release of ribonucleic acid from isolated rat liver nuclei. Biochem J 188:91–97.

Agutter, PS, Prochnow D (1994): Nucleocytoplasmic transport. Biochem J 300:609–618.

Agutter PS, McArdle HJ, McCaldin B (1976): Evidence for involvement of nuclear envelope nucleoside triphosphatase in nucleocytoplasmic translocation of ribonucleoprotein. Nature 263:165–167.

Agutter PS, McCaldin B, McArdle HJ (1979): Importance of mammalian nuclear-envelope nucleoside triphosphatase in nucleo-cytoplasmic transport of ribonucleoproteins. Biochem J 182:811–819.

Bernd A, Schroder HC, Zahn RK, Muller WE (1983): Nuclear-envelope nucleoside triphosphatase: Stimulation by poly(A)(+)mRNA and modulation by microtubule protein. Aktuelle Gerontol 13:119–121.

Chen M, Mason RP, Tulenko TN (1995): Atherosclerosis alters the composition, structure and function of arterial smooth muscle cell plasma membranes. Biochim Biophys Acta 1272:101–112.

Clawson GA, Koplitz M, Castler-Schechter B, Smuckler EA (1978): Energy utilization and RNA transport: Their interdependence. Biochemistry 17:3747–3752.

Clawson GA, James J, Woo CH, Friend DS, Moody D, Smuckler EA (1980): Pertinence of nuclear envelope nucleoside triphosphatase activity to ribonucleic acid transport. Biochemistry 19:2748–2756.

Clawson GA, Friend DS, Smuckler EA (1984): Localization of nucleoside triphosphatase activity to the inner nuclear envelope and associated heterochromatin. Exp Cell Res 155:310–314.

Czubryt MP, Ramjiawan B, Gilchrist JSC, Massaeli H, Pierce GN (1996a): The presence and partitioning of calcium binding proteins in hepatic and cardiac nuclei. J Mol Cell Cardiol 28:455–465.

Czubryt MP, Ramjiawan B, Pierce GN (1996b): The nuclear membrane integrity assay. Mol Cell Biochem (in press).

Dolphin PJ, Stewart B, Amy RM, Russell JC (1987): Serum lipids and lipoproteins in the atherosclerosis prone LA/N corpulent rat. Biochim Biophys Acta 919:140–148.

Dolphin PJ, Amy RM, Russell JC (1990): Effect of age on serum lipids and lipoproteins of male and female JCR:LA-corpulent rats. Biochim Biophys Acta 1042:99–106.

Gilchrist JSC, Pierce GN (1993): Identification and purification of a calcium-binding protein in hepatic nuclear membranes. J Biol Chem 268:4291–4299.

Kankaraj P, Singh M (1989): Influence of hypercholesterolemia on morphological and rheological characteristics of erythrocytes. Atherosclerosis 76:209–218.

Koletsky S (1973): Obese spontaneously hypertensive rats: A model for the study of atherosclerosis. Exp Mol Pathol 19:53–60.

Koletsky S (1975): Pathological findings and laboratory data in a new strain of obese hypertensive rats. Am J Pathol 80:129–142.

Kummerow FA, Przybylski R, Wasowicz E (1994): Changes in arterial membrane lipid composition may precede growth factor influence in the pathogenesis of atherosclerosis. Artery 21:63–75.

Kutryk MJB, Pierce GN (1988): Stimulation of sodium-calcium exchange by cholesterol incorporation into isolated cardiac sarcolemmal vesicles. J Biol Chem 263: 13167–13172.

Liu K, Pierce GN (1994): The modulation of membrane ion movements by cholesterol. In Bkaily G (ed): "Membrane Physiopathology." Norwell: Kluwer Academic Publishers, pp 291–317.

Markwell MK, Haas SM, Tolbert NE, Bieber LL (1981): Protein determination in membrane and lipoprotein samples: Manual and automated procedures. Methods Enzymol. 72:296–303.

McDonald JR, Agutter PS (1980): The relationship between polyribonucleotide binding and the phosphorylation and dephosphorylation of nuclear envelope protein. FEBS Lett 116:145–148.

Omodeo Sale F, Marchesini S, Fishman PH, Berra B (1984): A sensitive enzymatic assay for determination of cholesterol in lipid extracts. Anal Biochem 142:347–350.

Pelikanova T, Kohout M, Valek J, Base J, Stefka Z (1991): Fatty acid composition of serum lipids and erythrocyte membranes in type 2 (non-insulin-dependent) diabetic men. Metabolism 40:175–180.

Pierce GN, Dhalla NS (1983): Sarcolemmal Na+-K+-ATPase activity in diabetic rat heart. Am J Physiol 245: C241–C247.

Pierce GN, Kutryk MJB, Dhalla NS (1983): Alterations in Ca2+ binding by and composition of the cardiac sarcolemmal membrane in chronic diabetes. Proc Natl Acad Sci USA 80:5412–5416.

Pierce GN, Lockwood MK, Eckhert CD (1989a): Cardiac contracile protein ATPase activity in a diet induced model of noninsulin dependent diabetes mellitus. Can J Cardiol 5:117–120.

Pierce GN, Sekhon PS, Meng HP, Maddaford TG (1989b): Effects of chronic swimming training on cardiac sarcolemmal function and composition. J Appl Physiol 66:1715–1721.

Prochnow D, Thomson M, Schroder HC, Muller WEG, Agutter PS (1994): Efflux of RNA from resealed nuclear envelope ghosts. Arch Biochem Biophys 312:579–587.

Raess BU, Vincenzi FF (1980): A semi-automated method for the determination of multiple membrane ATPase activities. J Pharmacol Methods 4:273–283.

Ramjiawan B, Czubryt MP, Gilchrist JSC, Pierce GN (1996): Stimulation of rat nuclear nucleoside triphosphatase activity by cholesterol. J Cell Biochem (in press).

Russell JC, Ahuja SK, Manickavel V, Rajotte RV, Amy RM (1987): Insulin resistance and impaired glucose tolerance in the atherosclerosis-prone LA/N corpulent rat. Arteriosclerosis 7:620–626.

Russell JC, Koeslag DG, Amy RM, Dolphin PJ (1989): Plasma lipid secretion and clearance in hyperlipidemic JCR:LA corpulent rats. Arteriosclerosis 9:869–876.

Russell JC, Graham SE, Hameed M (1994): Abnormal insulin and glucose metabolism in the JCR:LA-corpulent rat. Metabolism 43:538–543.

Schroder HC, Nitzgen DE, Bernd A, Kurelec B, Zahn RK, Gramzow M, Muller WEG (1984): Inhibition of nuclear envelope nucleoside triphosphatase-regulated nucleocytoplasmic messenger RNA translocation by 9-b-D-Arabinofuranosyladenine 5'-triphosphate in rodent cells. Cancer Res 44:3812–3819.

Schroder HC, Rottmann M, Bachmann M, Muller WE (1986): Purification and characterization of the major nucleoside triphosphatase from rat liver nuclear envelopes. J Biol Chem 261:663–668.

Schroder HC, Rottmann M, Wenger R, Bachmann M, Dorn A, Muller WE (1988): Studies on protein kinases involved in regulation of nucleocytoplasmic mRNA transport. Biochem J 252:777–790.

Schroder HC, Wenger R, Ugarkovic D, Friese K, Bachmann M, Muller WEG (1990): Differential effect of insulin and epidermal growth factor on the mRNA translocation system and transport of specific poly(A+) mRNA and poly(A−) mRNA in isolated nuclei. Biochemistry 29:2368–2378.

Shinitzky M (1984): Membrane fluidity and cellular functions. In Shinitzky M (ed): "Physiology of Membrane Fluidity." Boca Raton, FL: CRC Press Inc., pp 1–51.

Sidransky H, Murty CN, Verney E (1984): Nutritional control of protein synthesis. Studies relating to tryptophan-induced stimulation of nucleocytoplasmic translocation of mRNA in rat liver. Am J Pathol 117:298–309.

Smith CD, Wells WW (1984): Solubilization and reconstitution of a nuclear envelope-associated ATPase. Synergistic activation by RNA and polyphosphoinositides. J Biol Chem 259:11890–11894.

Tokes ZA, Clawson GA (1989): Proteolytic activity associated with the nuclear scaffold. The effect of self-digestion on lamins. J Biol Chem 264:15059–15065.

Venkatraman JT, Clandinin MT (1988): Ribonucleic acid efflux from isolated mouse liver nuclei is altered by diet and genotypically determined change in nuclear envelope composition. Biochim Biophys Acta 940:33–42.