SUMMARY To investigate the pathophysiology of essential hypertension, detailed biochemical and clinical variables were collected and analyzed for 2091 Utah subjects aged 3 to 83 years. Three different measurements of erythrocyte cation transport were obtained: Na+-Li+ countertransport, Li+-K+ cotransport, and furosemide-insensitive Li+ efflux into MgCl2. Total plasma cholesterol, triglycerides, and high density lipoprotein cholesterol levels were obtained from fasting subjects. Levels of high density lipoprotein subfractions 2 and 3 were also obtained from 350 subjects. Standardized data collection also included blood pressure, height, weight, and presence or absence of a diagnosis or treatment of essential hypertension. In univariate analyses of all 1420 adults, each of the three transport systems showed the same significant correlations with triglyceride levels (r = 0.33-0.35, p < 0.0001), high density lipoprotein concentration (r = -0.19 to -0.21, p < 0.001), and weight (r = 0.22-0.28, p < 0.0001). In multivariate regression analyses, values for each transport system were significantly higher in hypertensive subjects; values for triglycerides, high density lipoprotein, and usually, the high density lipoprotein subfractions continued to have strong significant independent associations with all three transport systems; and weight remained significantly related only to Na+-Li+ countertransport. In separate logistic regressions, plasma triglyceride levels (positively, p < 0.001) and high density lipoprotein subfraction 3 levels (inversely, p < 0.03) were associated with hypertension itself. In multivariate analyses among 671 children, high density lipoprotein and high density lipoprotein subfraction 3 levels showed significant (p < 0.05) inverse correlations with Na+-Li+ countertransport and furosemide-insensitive Li+ efflux. These associations of all three cation transport systems with several blood lipids as well as with weight and hypertension suggest that a general relationship exists between blood lipids and membrane cation transport in the pathophysiology of essential hypertension. (Hypertension 8: 30-36, 1986)

KEY WORDS • triglycerides • high density lipoprotein cholesterol • Na+-Li+ countertransport • Li+-K+ cotransport • weight • hypertension • red blood cell membranes

OUABAIN-INSENSITIVE cation fluxes are being studied extensively because of their differing rates in hypertensive and normotensive subjects.1-4 The three transport systems discussed herein are Na+-Li+ countertransport, Li+-K+ cotransport, and furosemide-insensitive Li+ efflux into MgCl2. The Na+-Li+ countertransport system exchanges Na+ for Li+ (another Na+ in vivo) across the red blood cell membrane. The Li+-K+ cotransport consists of simultaneous outward transport of Li+ and K+ and seems to be related to the Na+-K+ cotransport system,5,6 although it should not be considered as an estimate for Na+-K+ cotransport. The Li+-K+ cotransport is calculated as the difference between the Li+ efflux into a solution containing MgCl2 with and without furosemide. We will refer to the furosemide-insensitive Li+ efflux into MgCl2 as a passive Li+ leak of the cell membrane, even though it subsequently may be found to include other transport mechanisms.

Several studies have found differences between hypertensive and normotensive subjects in these and other associated systems.1-5 A family history of hypertension, race, sex, and body size also have been reported to be associated with one or more of the transport systems6, 7,1-12; however, little is known about what causes the differences between these groups or what physiological factors may modify the transport systems. Since weight and body mass have been found to be related to these transport systems, we investigated the associations of different plasma lipids, including
Subjects and Methods

The sample for this study consisted of 2091 Utah subjects screened at the University of Utah Cardiovascular Genetics Research Clinic since 1980 in accordance with institutional guidelines. They are members or spouses of early coronary-prone, stroke-prone, and hypertension-prone families ascertained from the Utah population. The stroke pedigrees were ascertained by identifying all sibships from a computerized Utah state death certificate file for the years 1956 through 1975 that had had two or more stroke deaths before age 75 years. All descendants of these stroke subjects living within 50 miles of the clinic and giving consent to be studied, in addition to some of the descendants of the siblings of the stroke subjects, were included in the study. This involved two to four generations of descendants (n = 555). The coronary-prone pedigrees were similarly ascertained but required two or more deaths due to coronary heart disease before the age of 55 years (n = 942).

The hypertension pedigrees were ascertained from hypertensive subjects included in the Utah portion of the Hypertension Detection and Follow-up Program (HDFP) and from a random sample of probands found to be normotensive in the HDFP community screening (n = 594). The sampling of these pedigree types did produce a hypertension-prone sample as the age-specific and sex-specific hypertension incidence rates were higher than recently collected Utah population hypertension incidence rates (Unpublished data, 1985). In spite of the higher incidence rates in this sample, many nuclear families in the third and fourth generations have no hypertension and seem to be healthy in all respects. The almost 500 spouses of pedigree members are also more representative of the general population. Ninety-three percent of those invited into clinic were actually screened.

The study included 671 children (aged 3–17 years), 1277 normotensive adults (aged 18–83 years), and 143 hypertensive subjects (aged 24–77 years). The hypertensive subjects either were receiving antihypertensive treatment or had been told by a physician that their blood pressures were high, and the means of two diastolic blood pressure measurements were greater than 90 mm Hg when measured during a single visit to our clinic. Nearly all were receiving some form of thiazides, and many also were taking β-blockers. None were receiving calcium channel blockers or angiotensin converting enzyme blockers. Studies have shown little or no effect of thiazides and β-blockers on the transport systems, 3,5,8 although they can affect the lipid levels. To confound the lipid-transport relationship, however, the medications must influence both the lipids and the transport parameters.

The flux and clinical chemistry tests included in this study were not all performed at the start of clinic operation, which resulted in a different sample size available for each variable, as shown in Table 1. The HDL measurements were begun a few months after the clinic opening and were closely followed by triglyceride measurements. The HDL subfractions, HDL2 and HDL3, were measured only in the subjects belonging to the early coronary-prone kindreds and not in the general population. Ninety-three percent of those included were those who were actually screened.

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### Table 1. Means and Sample Sizes for Study Variables on 2091 Utah Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Children</th>
<th>Adults</th>
<th>Hypertensive adults</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>342:329</td>
<td>631:646</td>
<td>70:73</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>10.5±3.7</td>
<td>36.7±14.2</td>
<td>55.3±10.4*</td>
</tr>
<tr>
<td>Weight (lb)</td>
<td>79.9±34.6</td>
<td>161.0±35.3</td>
<td>179.4±40.9*</td>
</tr>
<tr>
<td>Mean BP (mm Hg)</td>
<td>68.2±9.5</td>
<td>84.1±10.1</td>
<td>97.6±11.7*</td>
</tr>
<tr>
<td>Cholesterol (mg/dl)</td>
<td>166.6±31.2</td>
<td>197.3±44.5</td>
<td>225.0±48.2</td>
</tr>
<tr>
<td>Triglyceride (mg/dl)</td>
<td>73.8±34.8</td>
<td>112.4±78.4</td>
<td>214.5±345.6*</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>50.0±10.6</td>
<td>48.7±12.5</td>
<td>48.0±12.6†</td>
</tr>
<tr>
<td>HDL2 (mg/dl)</td>
<td>13.5±6.6</td>
<td>11.6±8.6</td>
<td>9.2±7.1</td>
</tr>
<tr>
<td>HDL3 (mg/dl)</td>
<td>32.0±4.8</td>
<td>33.1±5.0</td>
<td>22.9±6.8†</td>
</tr>
<tr>
<td>Na⁺-Li⁺ countertransport (amol/L cells/hr)</td>
<td>233.9±81.9</td>
<td>267.9±108.2</td>
<td>338.3±134.9*</td>
</tr>
<tr>
<td>k_Li⁺-K⁺ (10⁻⁷/hr)</td>
<td>7.0±4.5</td>
<td>8.7±5.9</td>
<td>13.7±8.7‡</td>
</tr>
<tr>
<td>k_Li⁺ (10⁻⁷/hr)</td>
<td>17.1±5.6</td>
<td>15.5±5.3</td>
<td>20.6±8.0*</td>
</tr>
</tbody>
</table>

BP = blood pressure; HDL = high density lipoprotein cholesterol, k_Li⁺-K⁺ = rate constant for Li⁺-K⁺ countertransport.

* p < 0.0001, † p < 0.05, ‡ p < 0.001; analysis of covariance F test between the three groups adjusted for age and sex.
transport and Li⁺ leak determinations were begun in July 1983 in all participants.

Blood samples were drawn from supine, fasting participants from 0800 to 1000, and the fresh blood was transported on ice and analyzed for all flux and chemistry values except the HDL subfractions the same day. The Na⁺-Li⁺ countertransport was determined using the method of Canessa et al. modified as described by Smith et al. The Li⁺-K⁺ cotransport rate constant (k_{Li-K⁺}) and the Li⁺ leak rate constant (k_{Li-leak}) were determined as described by Smith et al. These rate constants were used since the intracellular Li⁺ concentration after Li⁺ loading was 6.0 ± 1.1 (SD) mmol/L of red blood cells and as these systems are not saturated at these Li⁺ concentrations. Cholesterol determination used a laboratory adaptation of an automated method, and the triglyceride procedure employed a coupled enzymatic method using commercial reagents (Cal Biochem, La Jolla, CA, USA). The HDL cholesterol measurements were quantitated using the Fe₃H₂SO₄ method, and the HDL subfractions were quantitated as described by Warnick and co-workers. Blood pressure was taken with the subjects in a sitting position with a random-zero mercury sphygmomanometer, and the mean of two readings was used. Mean blood pressure was calculated as diastolic pressure plus one-third pulse pressure.

Statistical analysis consisted of Pearson correlation coefficients and multivariate standardized regression coefficients to control for the associations of other variables with the transport systems. For each study variable shown in Table 1, initial tests for significant differences between the children, normotensive adults, and hypertensive adults were done using analysis of covariance to control for age and sex differences. Preliminary analysis of the data was sex-specific, age group-specific, and blood pressure group-specific since the relationships between the lipids and fluxes may have been different in each group.

Preliminary stratification was also important because the β-blocker and diuretic medication taken by many of the hypertensive subjects can affect lipid levels and most of the hypertensive subjects' blood pressure values are their treated blood pressure values. However, medication did not affect the lipid-transport system associations found in this study, as similar results were obtained when the data from hypertensive subjects were removed. A further concern was the presence of hyperlipidemia in some of the members of the coronary-prone kindreds. Therefore, similar analyses were done that were specific for each of the three kindred types. Each of the lipids was log-transformed, but the transformation did not alter the results or improve the fit of the regression line. Thus, the results using the untransformed values are reported.

Results

Table 1 gives the means of each variable for the three groups, and the significance levels indicate the probability from the analysis of covariance test between the three group means after adjusting for age and sex differences. The hypertensive subjects were significantly older than the normotensive adults (p < 0.0001). After adjustment for age and sex differences, the hypertensive subjects had significantly higher triglyceride concentrations than the normotensive adults (p < 0.0001). Although two hypertensive adults had triglyceride levels exceeding 1000 mg/dl, they are included in all analyses since excluding them had little effect on the estimates or significance tests. After correcting the p values in Table 1 for multiple comparisons, values for plasma cholesterol, HDL, and HDL subfractions were not significantly different among the three groups or between the hypertensive and normotensive adults. Significant differences were found among the three groups for Na⁺-Li⁺ countertransport, Li⁺-K⁺ cotransport, and Li⁺ leak: the hypertensive adults had higher values than the normotensive adults (all 3, p < 0.0001).

Associations in Adults

Table 2 gives the univariate and multivariate correlations for the lipids, weight, mean blood pressure, and hypertensive status with the three transport systems for all adults (normotensive and hypertensive). For each multiple regression model only one of the five lipids was entered at a time along with the other three covariates (weight, mean blood pressure, and hypertensive status). Since cholesterol, triglyceride, and HDL values had different effects on the other covariate coefficients, three standardized multivariate regression coefficients, three standardized multivariate regression coefficients for weight, mean blood pressure, and hypertensive status were given. They represent the association between the covariate and the transport system when cholesterol (top number of the 3), triglyceride (middle number), and HDL (bottom number) are used in the model. Because the slopes between the lipids and fluxes were not significantly different across sex and normotensive-hypertensive groups, these groups were combined in Table 2. To account for the mean differences between groups, however, a variable for hypertensive status was introduced into the model. With blood pressure group, weight, and mean blood pressure in the model, age and sex were no longer significant in any regression and were dropped from the equation. The associations changed little when data for the members of the coronary-prone kindreds or the hypertensive subjects, or both, were removed.

Plasma triglyceride level had a strong, consistent association with each of the three transport systems, both with (r = 0.22–0.28, p < 0.01) and without (r = 0.33–0.35, p < 0.0001) adjustment for other covariates, while HDL, HDL₁, and HDL₂ had consistent univariate and multivariate inverse associations with each transport system. The concentration of HDL₂ was uniformly inversely related to each of the fluxes, while that of HDL₃ was more strongly related to Li⁺ leak than to the other two transport systems. Plasma total cholesterol level was significantly associated only with Li⁺-K⁺ cotransport (r = 0.12, p < 0.05) and was not significant after adjustment for multiple significance tests.
The lipid associations with the three transport systems in the adults could not be explained by any of the covariates, nor could they explain the significantly higher cation flux rates found in the hypertensive subjects compared with those in the normotensive adults. The hypertensive-normotensive associations with Li+-K+ cotransport and Li+ leak were confounded by redundant information between that covariate and the covariates used in Tables 2 and 3, the standardized coefficients for the lipids were only slightly smaller than when the lipids were included one at a time for all three transport systems were calculated. The projected slopes for triglyceride versus Na+-Li+ countertransport (r = 0.20–0.24, depending on which lipid was included, p < 0.0001) but somewhat weaker than when the lipids were included one at a time for all three transport systems, and the blood pressure (r = 0.23–0.29, p < 0.01). The blood pressure-Li+ leak association was also strengthened after adjusting for other covariates.

Other Lipid Associations

The lipid values correlated with each other as expected. Values for HDL correlated with triglyceride values (r = 0.20) and with total cholesterol levels (r = 0.09), while triglyceride values correlated with total cholesterol values (r = 0.19; all 3, p < 0.001; all adults and children combined). When total cholesterol, triglyceride, and HDL values were included in the same regression model with the other two or three covariates used in Tables 2 and 3, the standardized coefficients for the lipids were only slightly smaller than when the lipids were included one at a time for any flux component. Therefore, plasma triglyceride and HDL appear to be independently associated with the fluxes.

To ensure that the correlation between the independent variables did not affect their estimated coefficients, the projected slopes for triglyceride versus the three transport systems were calculated. The projected slope represents the effect of a covariate (triglyceride) on the dependent variable (cation transport) after the redundant information between that covariate and the other covariates in the model has been removed from the other covariates by regression. The projected slopes for triglyceride versus Na+-Li+ countertransport, Li+-K+ cotransport, and Li+ leak in adults were...
0.35, 0.30, and 0.31, respectively (all, \( p < 0.0001 \)). These coefficients were higher than the multivariate coefficients in Table 2, which strengthens the conclusion that triglycerides are independently associated with all three transport rates.

**Lipids and Hypertension**

Since the plasma lipids were associated with hypertension-linked transport systems, logistic regressions of hypertensive-normotensive status on age, weight, sex, mean blood pressure, and one of the lipids were done separately for each lipid. Higher plasma triglyceride level (slope = 0.004 ± 0.001; \( p < 0.001 \)) was significantly associated with hypertension. Lower HDL level was also significant (slope = -0.07 ± 0.04, \( p < 0.009 \)), while HDL3 level was borderline. Cholesterol level was not significantly associated with hypertension. Although hypertensive medications could have affected this analysis of triglycerides and hypertension, the medications did not cause cholesterol level to become significant and may not have caused such a significant triglyceride association.

**Discussion**

Associations between plasma lipids and cell membrane ion fluxes are not surprising given that the membrane is composed of triglycerides, cholesterol, and phospholipids. Serum lipids may affect the membrane composition by altering the transport carrier position, changing the pore size and thereby affecting the passive leak, or making it more or less difficult for the ions to move through the lipid layers because of membrane fluidity or membrane charge distribution changes.

We have previously reported a small but significant total cholesterol level correlation with \( \text{Na}^+ - \text{Li}^+ \) countertransport in a subset of these data (\( n = 384 \)). Using this result, Worley et al.\(^{21} \) considered the possibility that cholesterol plays a role in the \( \text{Na}^+ - \text{Li}^+ \) countertransport seen during pregnancy and its gradual postpartum decrease. Their data suggest that the alterations in red blood cell membrane transport originate during erythropoiesis and remain during the life of the cell, since \( \text{Na}^+ - \text{Li}^+ \) countertransport decreases for 3 to 4 months after delivery rather than immediately, as does postpartum cholesterol concentration. Alterations in plasma lipid and lipoprotein levels could affect the amount or arrangement of these substances incorporated into the cell membrane during erythropoiesis.

Adragna et al.\(^{22} \) have recently reported on exercise-induced decreases in \( \text{Na}^+ - \text{Li}^+ \) countertransport accompanied by increased levels of HDL. They also found a similar correlation between plasma triglyceride levels and \( \text{Na}^+ - \text{Li}^+ \) countertransport (\( r = 0.30 \)), although no correlation was found between total cholesterol level and \( \text{Na}^+ - \text{Li}^+ \) countertransport. Thus, there is evidence from both a cross-sectional study and an experimental intervention study that plasma triglyceride and HDL levels affect the cellular ion transport rate.

Wiley and Cooper\(^{23} \) have shown that enriching the red blood cell membrane in vitro with cholesterol by using an incubating medium containing a high cholesterol/phospholipid mol ratio decreases the furosemide-sensitive \( \text{Na}^+ \) and \( \text{K}^+ \) influx. The cholesterol loading did not affect the furosemide-insensitive \( \text{Na}^+ \) and \( \text{K}^+ \) influx. Cooper et al.\(^{24} \) have also found that the incubating medium’s cholesterol/phospholipid mol ratio determined whether the cell membrane gained or lost cholesterol. The in vitro results indicated that an equilibrium is maintained between the serum cholesterol and the cholesterol in the cell membrane. For mol ratios of less than 1.0, the cell membrane loses cholesterol.

### References

1. Wiley and Cooper\(^{23} \)
2. Cooper et al.\(^{24} \)
3. Na+—Li+ countertransport\(^{20} \)
4. Furosemide-sensitive Na+ and K+ influx
5. Cholesterol loading
terol, which indicates that the cholesterol equilibrium also depends on the serum phospholipid concentration.

Our results show that plasma cholesterol was weakly associated only with Li⁺-K⁺ cotransport, which increased with an increasing plasma cholesterol concentration. If a direct relationship is assumed between plasma cholesterol level and Li⁺-K⁺ cotransport, then the results of Wiley and Cooper would imply that, with an increasing cholesterol level, there is either a greater increase in serum phospholipid concentration, a change in phospholipid fatty acid saturation or chain length, or perhaps a change in some other metabolite that alters the membrane fluidity, thereby actually removing cholesterol from the cell membrane and increasing the transport. Increased plasma triglyceride levels can increase phospholipid production in the liver. Since triglycerides had the strongest positive association with all three transport systems, this explanation may be feasible. However, phospholipid transfer to the cell membrane is much slower than cholesterol transfer.

Rather than an indirect plasma triglyceride–phospholipid concentration influence on cation transport, it is more probable that triglycerides directly affect the membrane. The data shown in Table 2 indicate that the triglycerides are associated with the rate of Li⁺ transport by the three transport systems, while the cholesterol coefficients are only significant for Li⁺-K⁺ cotransport. Increased lipolysis of triglyceride due to a change in phospholipid fatty acid saturation or chain length, or perhaps a change in some other metabolite that alters the membrane fluidity, could certainly contribute to a multifactorial picture of inheritance. The lipid and weight associations with Na⁺-Li⁺ countertransport and Na⁺-K⁺ cotransport are consistent in both the adult normotensive and hypertensive subjects and did not account for the significant difference between these two groups. The role of HDL may be an indirect one, controlling the other lipid concentrations that more directly affect the cell membrane. This possibility would help explain the consistent direction of the plasma lipid-cation transport associations and the plasma lipid-coronary heart disease associations. However, Adragna et al. found changes in HDL level and Na⁺-Li⁺ countertransport but no change in total cholesterol level, which indicates that HDL has a more direct role. Our cross-sectional study does not provide any direct evidence that any of the mentioned possibilities are occurring. More direct biochemical experiments are needed to determine how plasma triglyceride may affect ouabain-insensitive cation transport and how HDL may be involved.

Weight also had a positive association with the three transport systems in the adults. Other studies have also found a weight association with Na⁺-Li⁺ countertransport. Thus, another risk factor for both hypertension and coronary heart disease seems to be related to these methods of sodium transport. Plasma lipid levels and weight each contributed independently in their associations with the fluxes, especially with Na⁺-Li⁺ countertransport. The large inverse association between the membrane Li⁺ leak and weight found in children was surprising and suggests that some growth-related factor influences the membrane Li⁺ leak. As can be seen in Table 1, the children had higher Li⁺ leak values than did the normotensive adults. Plasma triglyceride level also was less strongly related to the transport systems in children than in the adults. Only HDL and its subfractions retained much of their association with cation transport in the children.

The finding that multiple risk factors associated with hypertension and coronary heart disease are related to the rates of these transport systems agrees with the results of a genetic analysis of Na⁺-Li⁺ countertransport. The Na⁺-Li⁺ countertransport, as measured in large pedigrees, was found to follow a polygenic transmission pattern or, possibly, a mixed model with a small major gene effect. The polygenic heritability was estimated as 71%. Lipid and weight effects along with the other genetic and physiological controls of membrane constitution and metabolism could certainly contribute to a multifactorial picture of inheritance.

The lipid and weight associations with Na⁺-Li⁺ countertransport, Na⁺-K⁺ cotransport, and furosemide-insensitive Li⁺ efflux found in this study should provide additional clues to the pathophysiology and heterogeneity of hypertension and these cation transport systems and why they differ among populations, races, and diagnostic categories.

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