Genetic and Environmental Components of Thyroxine Variation in Mennonites from Kansas and Nebraska

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Abstract Thyroxine is an endocrine hormone that regulates cellular and organismic metabolism. Current research on thyroxine has primarily examined its adaptive potential and genetic inheritance patterns. To date, no studies have attempted to investigate the interaction between the genetic and environmental components of thyroxine variation. This approach is useful because hormones are on feedback regulation; thus interaction occurs between the environment and gene expression. The purposes of this research are to characterize the genetic and environmental components of thyroxine variation using univariate statistics and to estimate the genetic and cultural heritabilities through path analysis. For univariate analyses, analyses of variance are used to determine whether or not age, sex, or community affiliation are covariates of thyroxine level. Significant differences existed in thyroxine level based on sex and community affiliation ($p < 0.05$). The genetic and environmental components of thyroxine variation were partitioned through path analysis. Heritability was estimated at 0.317 ± 0.109 for the genetic component and at 0.060 ± 0.029 for the environmental component. The environmental variables that contributed to the variation in thyroxine level were caffeine consumption, blood calcium level, and biceps skinfold thickness.

Thyroxine is a hormone in the endocrine system that regulates cellular and organismic metabolism and governs calorigenesis, lipid, carbohydrate, and protein metabolism, respiratory and cardiovascular function, and growth and development (Butt 1975; Hardy 1981). There are two forms of this hormone: thyroxine (T_4) and triiodothyronine (T_3). T_3 is recognized as the metabolically active form of thyroxine (Butt 1975; Hardy 1981). Compared to T_3, T_4 is secreted at a ratio of 20 to 1 and is less responsive to environmental influences (Harland and Orr 1975; Hardy 1981; Danforth 1986). Current research on thyroxine focuses primarily on its metabolic functions, although a few studies have examined its adaptive potential (Schwartz 1983; Riis and Madsen 1985;

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Ballard 1986; Legrand 1986; Pimentel 1987; Cabello and Wrutniak 1990; Maruo et al. 1992a,b; Sower et al. 1992; Facchini et al. 1997). For example, thyroid hormone fluctuations have been reported in cold adaptation studies and a relationship between thyroxine and physique has been suggested (Reed et al. 1986, 1990, 1992; Silverin et al. 1989; Gaikwad et al. 1990; Kwiecinski et al. 1991; Rone et al. 1992; Kirchengast 1994).

To date, no studies have attempted to explain thyroxine variation by focusing on the interaction between genetic, environmental, and endocrinological factors. However, integrating information from several disciplines is particularly informative when studying hormones such as thyroxine because of their complex interrelationships. It is important to identify both the genetic and the environmental components of normal thyroxine variation because thyroxine variation is due to both genetic and environmental factors. The goals of this research are to examine the genetic and environmental components involved in thyroxine variation using univariate statistics and to estimate the genetic and cultural heritabilities using path analysis.

Materials and Methods

Population. The data for this study were collected from three Mennonite communities, two in Kansas (Goessel and Meridian) and one in Nebraska (Henderson) (Figure 1), as part of a multidisciplinary study of biological aging. The collected data included demographics, reproductive histories, blood specimens, nutritional data, anthropometrics, genealogies, psychological questionnaires, and neuromuscular tests (Crawford and Rogers 1982). Crawford and Rogers (1982) demonstrated that Goessel and Henderson are most similar genetically because they both originated from a single community, Alexanderwohl, during the 1870s. In contrast, Meridian is genetically heterogeneous because it was recently founded by a charismatic leader and consisted of several familial groupings of various origins.

Data Collection and Analysis. From November 1980 to January 1981 data were collected from 1062 Mennonites, ranging in age from 18 to 94 years, from the 3 communities (Goessel, Henderson, and Meridian). Individuals who had a history of thyroid disease were excluded from the analysis, thus reducing the sample used in this study to 1042 individuals (Goessel, 460 individuals; Meridian, 85 individuals; Henderson, 497 individuals). The total sample of 1042 individuals was included in the computation of univariate statistics. However, only family data were used for the path analysis, thus reducing the sample to 378 individuals representing 112 families (111 fathers, 112 mothers, and 155 children).

To assess thyroxine levels, we drew blood specimens from nonfasting volunteers. The serum T_4 levels were assayed using the standard radioim-

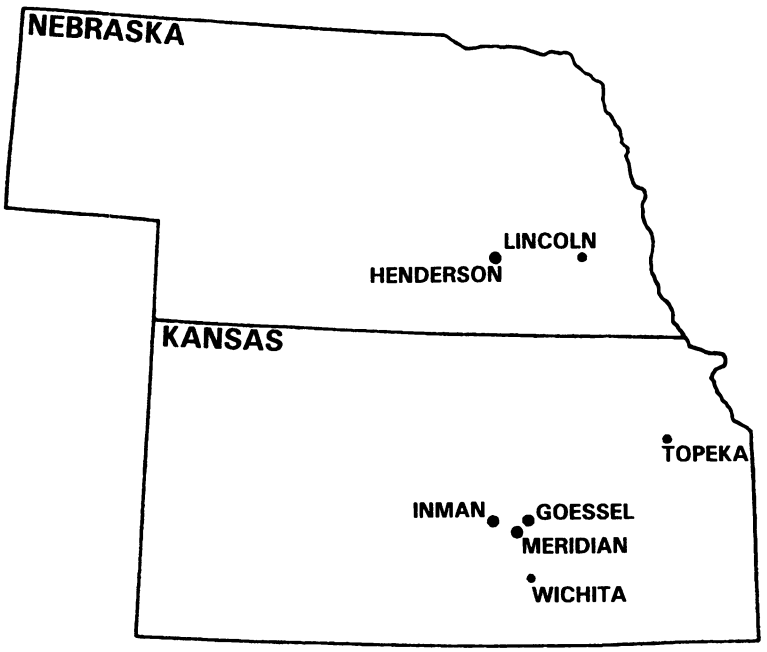

Figure 1. Locations of the Mennonite communities of Goessel, Meridian, and Henderson in Kansas and Nebraska.

munoassay technique of Consolidated Biomedical Laboratories (Roche Labs, Wichita, Kansas) immediately after the blood was drawn.

Univariate Methods. Univariate methods were used to examine population and individual differences. For population level analyses means, standard deviations, and standard errors of thyroxine levels for the three Mennonite communities were compared to each other and to the normal physiological variation. For individual level analyses age effects were examined by regressing thyroxine variation on age and on age-squared. Sex differences in thyroxine level were analyzed using a one-way analysis of variance (ANOVA). Furthermore, thyroxine levels for females were partitioned into pre- and postmenopausal groups and were compared using ANOVA. Last, the postmenopausal women were separated into those who had complete hysterectomies (surgical menopause) versus those who had experienced menopause without hysterectomies (natural menopause), and the thyroxine levels were compared using ANOVA.

Analytical Methods. Path analysis was used to estimate the genetic and environmental components of thyroxine variation by partitioning the two

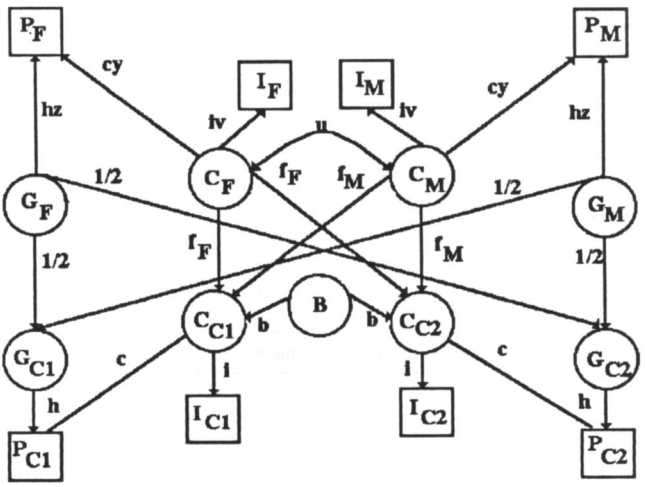

Figure 2. Path diagram of PATHMIX III demonstrating the relationship between parents and offspring with respect to genotype and environment. The subscripts, F, M, C1, and C2, represent father, mother, and two children. *P* is phenotype, *G* is genotype, *C* is transmissible environment with index *I*, and *B* is nontransmitted common sibship environment (Rao, Williams et al. 1983).

components on the basis of nuclear family data by using a predetermined causal scheme, the path diagram (see Figure 2) (Wright 1918; Rao et al. 1982). This analysis uses PATHMIX III, a computer program designed by D.C. Rao and colleagues to directly use nuclear family data (Rao et al. 1982; Rice et al. 1991).

A cultural or environmental index was created using a phenotype defined by thyroxine level to characterize the familial environment (Morton 1974; Rao, Williams et al. 1983). To construct this index, we first used linear regression to correct the thyroxine level for age and sex effects. The age- and sex-corrected data were then normalized by the inverse normal transformation and were used as the phenotype values. Once the phenotype was characterized, an environmental index was constructed by multiple regression analysis of the phenotype (forward and backward and best subsets). The following variables were used to determine the significant contributors to T_4 variation: blood glucose, low-density lipoprotein, blood calcium, smoking, alcohol consumption, caffeine consumption, church affiliation, weight, height, and subscapular, triceps, biceps, and suprailiospinal skinfold thicknesses. The regression equation ($Y = aX + b$) produces a line that defines the relationship between the independent variables (possible contributors) and the dependent variable (thyroxine level). The Y's from the regression equation are called the predicted Y's. The normalized predicted Y's constitute the cultural index.

Table 1. Definition of the Ten Parameters in the Path Model (Rao, Williams et al. 1983)

Parameter	*Definition*
h	Effect of genotype on child's phenotype (square root of genetic heritability)
h_z	Effect of genotype on adult's phenotype
c	Effect of environment on child's phenotype (square root of cultural heritability)
c_y	Effect of environment on adult's phenotype
u	Correlation between parental environments
b	Effect of sib-transmitted common sibship environment on child's environment
f_F	Effect of father's environment on that of a child he rears
f_M	Effect of mother's environment on that of a child she rears
i	Effect of environment on child's index
i_V	Effect of environment on adult's index

PATHMIX III uses the following linear equation to estimate the various parameters:

$$P = G + C + R, \tag{1}$$

where P is the phenotype, G is the genetic contribution, C is the cultural and familial environmental contribution, and R is the residual resulting from random environmental factors (Rao, Williams et al. 1983; Friedlander et al. 1986). It is assumed that G, C, and R are uncorrelated (Rao, Williams et al. 1983). The "structural equation" is produced when the variables in Eq. (1) are standardized and are all divided by the variance of the phenotype (Rao, Morton et al. 1983; Rao, Williams et al. 1983):

$$p = hG^* + cC^* + rR^*, \tag{2}$$

where G^*, C^*, and R^* are the standardized variables, $h = (V_g/V_p)$, $c = (V_c/V_p)$, and $r = (V_r/V_p)$, and V is the variance. These are also known as the standardized partial regression coefficients or path coefficients. Dividing both sides of the structural equation by the phenotypic variance yields (Rao, Morton et al. 1983; Rao, Williams et al. 1983)

$$1 = h^2 + c^2 + r^2, \tag{3}$$

where h^2 is the genetic heritability, c^2 is the cultural heritability, and r^2 is the residual variation. This mathematical expression is known as the equation for complete determination (Rao, Morton et al. 1983; Rao, Williams et al. 1983).

The PATHMIX III method contains 6 known variables (P_F, phenotype of father; P_M, phenotype of mother; P_C, phenotype of child; I_F, index of father; I_M, index of mother; I_C, index of child) and 10 unknown parameters, which are displayed in the path diagram (Figure 2). Table 1 defines each of the 10 unknown parameters that were estimated by maximum likelihood. The 6 known variables yielded 15 correlations, several of which are repetitive (P_F, I_F and P_M, I_M and P_F, I_M and I_F, P_M), thus reducing the number of correlations

Table 2. Definitions of the Null Hypotheses Tested

Null Hypothesis	*Mathematical Expression*
No intergenerational differences with respect to heritabilities	$y = z = 1$
No marital resemblance (assortative mating)	$u = 0$
No common sibship effect	$b = 0$
Equal parental transmission of familial environment	$f_F = f_M$
No genetic heritability	$h = z = 0$
No cultural heritability	$c = y = 0, i = v = 1$

to 13. Moreover, the 3 sib-sib correlations raise the total number of correlations to 16, which serve as a basis for the interpretation of the data and a test of the fit of the general and most parsimonious models (Rao et al. 1984).

PATHMIX III estimates the model directly from the family data instead of by computing the model from the correlations. However, the 16 correlations, 6 means, and 6 variances of the 6 variables are used to test the overall log-likelihood function (Rao et al. 1984; Duggirala and Crawford 1995). To test the functions, we calculated the log-likelihood for single nuclear families using the deviation of the phenotype from the mean; then the overall log-likelihood was calculated using the log-likelihood from all nuclear families.

The overall likelihood ratios were used to test the goodness of fit of given models and to test specific hypotheses. Twice the difference in likelihood ratios follows a chi-square distribution. To test the fit of the model using all the path coefficients and their variances, we tested the log-likelihood of the correlation model against the log-likelihood of the unconstrained path model. To test the null hypotheses listed in Table 2, we tested the log-likelihood for each constrained model against the log-likelihood for the unconstrained path model. The degrees of freedom for all tests was the difference in the number of estimated parameters.

Results

Univariate Analysis. Means and standard deviations of thyroxine levels in the three Mennonite communities are presented in Table 3. The mean values of thyroxine level in these populations fall within normal physiological variation (Hardy 1981). The distribution of thyroxine level appears to be relatively normal but with a slight skew to the left (Figure 3). No statistically significant age effects were observed in the distribution of the T_4 levels. However, one-way ANOVA revealed significant differences between the communities and the sexes ($p < 0.05$) (Figure 4). Moreover, thyroxine levels of pre- and postmenopausal women were significantly different ($p < 0.05$) with a mean thyroxine level for the postmenopausal group of 8.38 versus 8.95 for

Table 3. Means and Standard Deviations of Thyroxine Levels in the Three Mennonite Communities

Community	*N*	*Mean*	*SD*
Goessel			
Males	220	8.020	1.583
Females	240	8.524	1.445
Meridian			
Males	38	7.826	2.079
Females	47	8.085	1.926
Henderson			
Males	242	8.289	1.589
Females	255	8.707	1.711

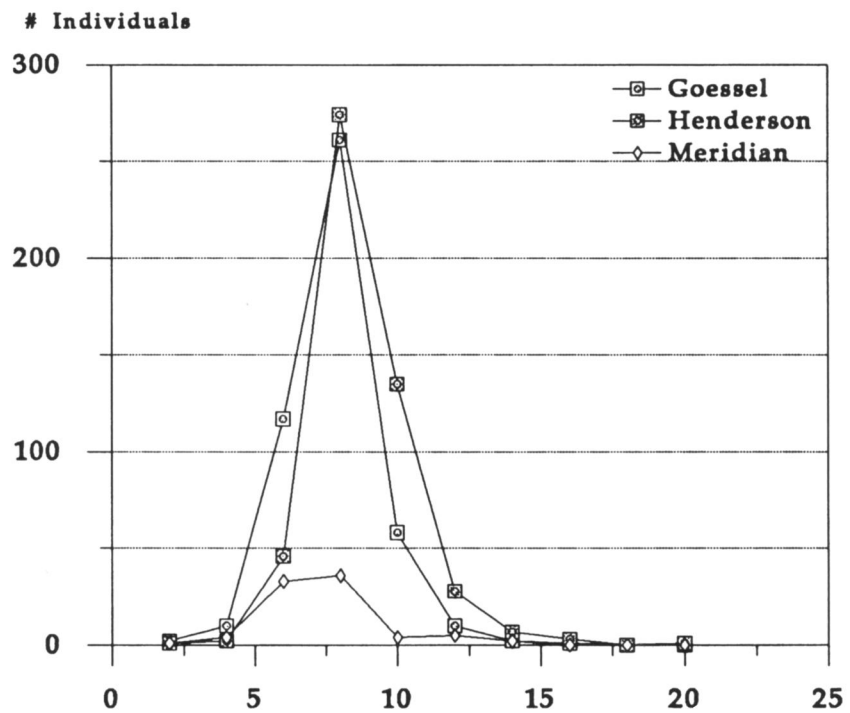

Figure 3. Frequency distributions for thyroxine levels in the three Mennonite communities.

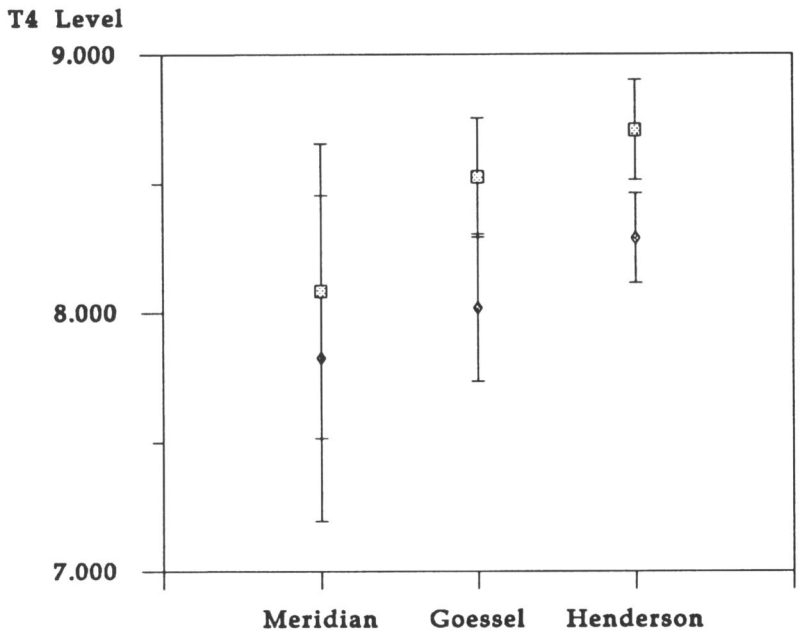

Figure 4. Mean thyroxine (T_4) levels and standard error bars in men (diamonds) and women (squares) for the three Mennonite communities.

the premenopausal group. Yet no statistical difference was detected between the women who had hysterectomies and those who had experienced natural menopause.

Path Analysis. Using the criteria of the F ratios, adjusted R^2, mean square error, tolerance, and Mallow's C_p, we constructed the environmental index from the best subsets of the regression of all possible variables. This model contained three variables: caffeine intake, blood calcium level, and biceps skinfold thickness. The familial correlations estimated by maximum likelihood are found in Table 4. Only the father-child phenotype correlation was significant, suggesting a paternal effect. For the environmental index correlations the mother-father and child-child correlations were highly significant, whereas the other index correlations were low, suggesting intergenerational differences.

Parameter estimates for the general model, a summary of the null hypotheses tested, and parameter estimates for the most parsimonious model are found in Tables 5 through 7. The general (10-parameter) model fits the data ($\chi^2_6 = 6.627, p = 0.36$) (Table 5).

The most parsimonious model was generated from the accepted null hypotheses, which include the following hypotheses: equal parental trans-

Table 4. Familial Correlations Estimated by Maximum Likelihood

Variable	*Correlation*	*n*	*p*
Phenotype			
P_F, P_M	−0.0377	94	ns
P_F, P_C	0.3052	144	$p < 0.01$
P_M, P_C	−0.0177	138	ns
P_{C1}, P_{C2}	0.1258	40	ns
Index			
I_F, I_M	0.4240	111	$p < 0.01$
I_F, I_C	0.2281	121	$p = 0.01$
I_M, I_C	0.3144	119	$p < 0.01$
I_{C1}, I_{C2}	0.1957	25	$p < 0.01$
Cross traits			
P_F, $I_F = P_M$, I_M	0.2357	207	
P_F, $I_M = I_F$, P_M	0.0788	188	
P_F, I_C	0.0986	129	
I_F, P_C	0.0423	125	
P_M, I_C	0.0595	146	
I_M, P_C	0.0843	117	
P_{C1}, I_{C2}	−0.1491	55	
P_{C2}, I_{C1}	0.2243	118	

mission of environment, no generational differences, and no common sibship. This model was compared for goodness of fit to the observed (Table 4) and the expected (general 10-parameter; Table 5) models using a chi-square statistic. The most parsimonious model estimated the genetic heritability as 0.317 (0.109) and the cultural heritability as 0.060 (0.029).

Table 5. Parameter Estimates and Their Standard Errors for the General Model

Parameter	*Estimate*	*Standard Error*
h	0.6201	0.2452
c	0.2567	0.0817
y	0.9206	0.3934
z	0.8175	0.6481
u	0.4238	0.2128
f_F	0.1357	0.0980
f_M	0.2402	0.0982
b	0.3571	0.2617
i	1.0000	0.0000
v	1.0000	0.2377
χ^2	12.625	
d.f.	6	
p	0.0500	

Table 6. Chi-Square Values and Probabilities Associated with the Tested Null Hypotheses

Null Hypothesis	*d.f.*	χ^2	*Probability*
$y = z = 1$	2	0.092	0.955
$u = 0$	1	18.949	<0.0001
$b = 0$	1	0.452	0.5014
$f_F = f_M$	1	0.460	0.4976
$h = z = 0$	2	8.373	0.0152
$c = y = 0, i = v = 1$	4	19.221	0.0007
Parsimony			
$f_M = f_F, y = z = 1, b = 0$	4	0.980	0.928

Discussion

Univariate Analysis. The significant differences in thyroxine level that exist between the three communities may be attributed to either environment or genetics. Considering that the three Mennonite communities share similar diets and lifestyles, it is unlikely that these differences are due to environment. Furthermore, Henderson and Goessel are more similar to each other with respect to mean thyroxine level than to Meridian (Figures 3 and 4). Indeed, Goessel and Henderson were once a single community that later divided. Because these communities are historically related, they should be more similar genetically. Even though biological differences exist between the com-

Table 7. Parameter Estimates for the Most Parsimonious Model

Parameter	T_4[a]	*Standard Error*
h	0.5630	0.0928
c	0.24434	0.0597
y	(1.0000)	(0)
z	(1.0000)	(0)
u	0.4217	0.1865
b	(0.0000)	(0)
f_F	(0.2000)	(0)
f_M	(0.2000)	(0)
i	1.0000	0.0000
v	1.0000	0.2029
χ^2	0.980	
d.f.	4	
p	0.928	

a. Values in parentheses represent variables that were fixed.

munities, these differences do not affect the results of path analysis because these methods are concerned with the segregation of traits within families, not within populations.

Path Analysis. Path analysis was used to partition the genetic and environmental contributions to variation in thyroxine level. Because the environment cannot be directly measured (Rao, Williams et al. 1983), an index was developed using the significant contributors to thyroxine variation. This index identifies which environmental factors were significant. In this analysis blood calcium level, caffeine consumption, and biceps skinfold thickness contributed significantly to the expression of thyroxine level. The genetic component of path analysis yielded heritability estimates and information on intergenerational differences, homogamy, and other factors.

Correlation analysis revealed that a significant positive relationship exists between caffeine intake and thyroxine level. To date, there has been no specific study of the relationship between thyroxine and caffeine; however, there is evidence for a possible interaction between these variables. Previous studies have demonstrated that thyroid hormones increase the level of adenylate cyclase activity and the cellular cyclic AMP (cAMP) level (Segal et al. 1985, 1989). cAMP is a secondary messenger for the action of many hormones. When hormones attach to their receptors on the plasma membrane, cAMP provides the necessary energy for the hormonal action (Stryer 1988). Evidence for cAMP as a secondary messenger includes stimulation of adenylate cyclase and positive correlations between cellular cAMP concentrations and hormonal concentrations (Stryer 1988). Segal et al. (1985, 1989) demonstrated that increases in thyroxine cause concomitant increases in the adenylate cyclase activity and cAMP levels, suggesting that thyroxine uses cAMP as a secondary messenger. Caffeine acts synergistically with hormones that use cAMP as a secondary messenger (Stryer 1988). Because of this nonadditive effect, positive correlations between thyroxine and caffeine level would be expected. Thus our data support a synergistic action of caffeine and thyroxine through cAMP.

Previous studies identified a relationship between thyroxine and calcium level (Davis et al. 1983; Segal et al. 1985, 1989). Segal et al. (1989) suggested that this relationship is due to calcium's regulation of thyroxine activity. This hypothesis is based on two previous findings: (1) T_3 and T_4 stimulation of Ca^{2+} ATPase activity depends on baseline levels of cytoplasmic calcium because the calcium is pumped out of the cell by Ca^{2+} ATPase (Davis et al. 1983; Segal et al. 1985, 1989); and (2) calcium is required for the stimulation of cAMP and adenylate cyclase by thyroxine (Segal et al. 1989). Thus calcium regulates thyroxine's activity because intracellular calcium is necessary for thyroxine's effects on cAMP and Ca^{2+} ATPase. When thyroxine levels are elevated, Ca^{2+} ATPase is stimulated and calcium moves into the bloodstream, which in turn lowers the intracellular calcium levels. When the intracellular

levels of calcium become too low, thyroxine fails to stimulate cAMP and adenylate cyclase, thereby diminishing thyroxine's hormonal effects. Therefore calcium concentration is a regulator of thyroxine activity through Ca^{2+} ATPase. However, thyroxine can also regulate blood calcium levels by releasing calcium from the cells. Calcium cannot exit the cell without the pumping action of Ca^{2+} ATPase.

The relationship between calcium and thyroxine may explain why postmenopausal women are particularly prone to osteoporosis. There is a significant difference in pre- and postmenopausal women's thyroxine levels, with postmenopausal women having lower levels. After menopause there is less thyroxine in the bloodstream; thus less thyroxine enters the cells and less calcium is released by means of the Ca^{2+} ATPase pump. The body continues to require the same level of calcium, and therefore calcium is removed from bone. Indeed, the relationship between calcium and thyroxine can be seen as one maintaining an equilibrium. At menopause the equilibrium is disrupted by a drop in thyroxine level. Gradually, the body adjusts to a lower thyroxine level and the rate of bone loss is reduced. This apparent relationship is supported by previous research that demonstrated that the rate of bone loss is greatest during the first five to ten years after menopause, after which the rate declines (Krolner and Nielsen 1982; Geusens et al. 1986).

Biceps skinfold thickness is positively correlated with thyroxine level and may be explained through energy balance. During periods of positive energy balance and surplus energy, fat is deposited. Indeed, Kirchengast (1994) found positive correlations between the thyroid hormones and various measures of body fatness. Individuals with prolonged positive energy balance have higher thyroxine levels because additional thyroid hormone is required to metabolize the surplus food intake. Extremely low thyroxine levels also promote fat storage but only in regions of primary fat storage such as the abdomen, hips, and buttocks (Van Hardeveld 1986; Després et al. 1988). Biceps skinfold thickness is the best predictor of thyroxine level because the biceps maintain a significant portion of muscle in most individuals and thus are less likely to be a storage area for fat (Després et al. 1988; Lohman 1992). Therefore the relationship between thyroxine level and biceps skinfold thickness may be due to energy balance.

Heritability Estimates. Unlike the univariate methods, path analysis estimates the genetic and cultural heritabilities (which were 0.317 and 0.060, respectively) for Mennonite thyroxine levels. These estimates explain only 38% of the observed variation, and it is unclear what causes the other 62% of the thyroxine variation. The genetic heritability measured by path analysis is slightly higher than the univariate estimate of heritability for the Mennonites (Walawender and Crawford 1992), slightly lower than the estimate for Salt Lake City male twins (Meikle et al. 1988), and similar to heritabilities measured by variance decomposition for Mexican Americans (Comuzzie et

al. 1996). The variation in genetic heritability estimates is due to several factors. First, the data types are different; the Salt Lake City probands are twins, which can inflate estimates. Second, the methods of analysis can alter the heritability estimates. Path analysis splits variation into genetic and environmental components, variance decomposition separates the genetic variation into the additive and dominance components, and univariate methods examine only additive genetic variation. Therefore slight differences in estimates are to be expected. Third, the heritability estimates are population specific; thus intrapopulation variation is normal.

Although the genetic heritability estimates are well within the range of previous studies, a large residual remained. One possible reason for this residual is the confounding effect of menopause. Although no significant age effects were observed, there was a significant menopausal effect. Postmenopausal women had significantly lower thyroxine levels than did premenopausal women. This is problematic when estimating heritabilities because there are two distinct categories of women. A simple correction for sex may oversimplify the physiological situation by forcing females into a single category so that neither pre- nor postmenopausal females will be comparable to males or to each other. Attempts to correct for the menopausal effect were made within females; however, these corrections resulted in insignificant general models of path analysis. This could be due to the dramatic changes that occur during perimenopause and immediately after menopause.

Conclusions

In this study we estimated the genetic and environmental components of thyroxine variation. There are significant sex effects on thyroxine levels that must be corrected in any studies that include both sexes. Although no age effect was observed, a menopausal effect was detected. Future studies must address the question of how to statistically correct for menopausal status. Dividing the female sample into pre- and postmenopausal groups is insufficient because physiological changes commence several years before menopause during the period of perimenopause. Thus corrections must account for perimenopausal changes in addition to menopause.

Thyroxine level was influenced by caffeine intake, blood calcium level, and biceps skinfold thickness. Through an examination of the relationship of each of these variables to thyroxine, a better understanding of the variation in thyroxine levels is gained. Caffeine appears to act synergistically with thyroxine by means of cellular cAMP. Calcium regulates thyroxine activity by controlling thyroxine's entrance into the cell. Furthermore, the thyroxine-calcium relationship may explain why osteoporosis occurs after menopause. Last, biceps skinfold thickness may demonstrate how thyroxine is influenced by the long-term energy balance. Thus, by examining the variables that affect

thyroxine variation, we can gain a better understanding of the inheritance of thyroxine.

To date, no previous research has estimated the cultural component of thyroxine variation. However, by examining the residual value for this model, we can assume that other factors (not taken into consideration) affect thyroxine level variation. For example, estrogen levels have been demonstrated indirectly to play a significant role in thyroxine variation (Chopra 1981; Sower et al. 1992). Unfortunately, estrogen levels were not available for this data set; thus the relationship between estrogen and thyroxine could not be examined directly. Future research could address how permanent changes in estrogen levels, such as at menopause, affect thyroxine variation and other biochemical levels. Indeed, the key to understanding thyroxine variation may be through an examination of the complex interactions of the endocrine system.

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