Colloquium: Homocyst(e)ine, Vitamins and Arterial Occlusive Diseases

The Hordaland Homocysteine Study: The Opposite Tails Odds Ratios Reveal Differential Effects of Gender and Intake of Vitamin Supplements at High and Low Plasma Total Homocysteine Concentrations[1]

HELGA REFSUM,*[2] OTTAR NYGÅRD,† GUNNAR KVÅLE,† PER MAGNE UELAND* AND STEIN EMIL VOLLSET†

*Department of Clinical Biology, Division of Pharmacology, †Section for Medical Informatics and Statistics, and ‡Centre for International Health, University of Bergen, Bergen, Norway

ABSTRACT Both female sex and intake of vitamin supplements are known to be associated with a low mean plasma homocysteine level. In the present study, we used multiple logistic regression analyses to investigate the relation between these two variables and plasma homocysteine at the extreme ends of the plasma homocysteine distribution curve. We propose that the obtained set of odds ratios for hyper- and hypohomocysteinemia, referred to as the opposite tails odds ratios, may be an alternative approach to study determinants of plasma homocysteine. J. Nutr. 126: 1244S–1248S, 1996.

INDEXING KEY WORDS:

homocysteine • vitamins • gender • correlations

Both retrospective (Kang et al. 1992, Malinow and Stampfer 1994, Ueland et al. 1992) and prospective (Arnesen et al. 1995, Stampfer et al. 1992) studies have demonstrated a relation between moderate hyperhomocysteinemia and premature vascular disease in the coronary, cerebral and peripheral arteries. Most studies conclude that plasma homocysteine is an independent risk factor for cardiovascular disease (Boushey et al. 1995, Kang et al. 1992, Malinow and Stampfer 1994, Selhub et al. 1995), but an association between homocysteine levels and established cardiovascular risk factors such as serum cholesterol (Araki et al. 1989, Kang et al. 1986), blood pressure (Araki et al. 1989, Malinow et al. 1989) or cigarette smoking (Arnesen et al. 1995, Bergmark et al. 1993) has occasionally been demonstrated. Recognized determinants of total plasma homocysteine include inherited characteristics such as sex and the functional state of enzymes related to homocysteine metabolism, whereas age, cobalamin or folate status and renal function are among the acquired causes (Ueland et al. 1992). Thus, differences in plasma homocysteine levels in various subject populations can usually be attributed to one or more of these factors.

The relation between determinants of plasma homocysteine has usually been studied by using regression models with plasma homocysteine as the dependent variable. The multiple linear regression technique determines the relation between a set of independent variables and the mean of a normally distributed dependent variable. Thus, this model implies that the independent variables have the same effect on high and low values of the dependent variable (Altman 1991). Biologically, however, it is possible that a factor selectively influences extreme high or low values of homocysteine with only moderate effect on the overall mean. Such effects can be overlooked with linear regression but may be studied with a set of logistic regression analyses, each at a different cut point for the dichotomous dependent variable.

Recently, University of Bergen in cooperation with

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[2] To whom correspondence should be addressed: Department of Clinical Biology, Division of Pharmacology, Armauer Hansens hus, 5021 Bergen, Norway.

the National Health Screening Service of Norway performed a cross-sectional study on plasma homocysteine in about 18,000 subjects aged 40–67 y from the County of Hordaland (Nygård et al. 1995). In a subgroup of this population (n = 11,425), we used a multiple logistic regression model to calculate the odds ratios (OR)[3] for hyper- and hypohomocysteinemia and thereby demonstrated that the relation between plasma homocysteine and a given factor may change along the distribution of plasma homocysteine.

MATERIALS AND METHODS

Study population. From April 1992 to April 1993, a total of 18,043 subjects, aged 40–67 y, from Hordaland County in Western Norway participated in an ongoing (Bjartveit et al. 1979) national health screening study. Data, including information about age, gender, intake of vitamin supplements, smoking habits and coffee consumption were collected through questionnaires. In the present study, the statistical analyses were limited to subjects in the age groups 40–42 y (n = 9115) and 65–67 y (n = 2310), who reported that they were without a previous diagnosis of coronary heart disease, cerebrovascular disease, hypertension or diabetes, and from whom data about intake of vitamin supplement were obtained, i.e., a total of 5134 men and 6291 women.

Intake of vitamin supplements was categorized into five groups according to use during the year and frequency of intake during the week. The lowest category included subjects who never used vitamins (n = 3876), whereas the highest category represented those who took vitamins 6–7 d a week during the whole year (n = 1321). In a subpopulation (n = 235), there was a significant correlation between the categories for intake of vitamin supplements and the plasma levels of folate (r = 0.24), also after adjustment for important determinants of plasma homocysteine (see below). There was no relation between vitamin supplement intake and plasma cobalamin.

Blood sample collection and homocysteine determination. Plasma was prepared from whole blood collected into an evacuated tube containing ethylene diamine tetraacetate. The tube was placed in a refrigerator (4–5°C) within 30 min and centrifuged within 3 h. The plasma fraction was then transferred to plastic vials, sent to our laboratory by mail and then stored frozen (−20°C) until the homocysteine analysis was performed.

Total plasma homocysteine was determined using a modification of a fully automated assay based on precolumn derivatization with monobromobimane followed by reversed phase high performance liquid chromatography (Fiskerstrand et al. 1993, Refsum et al. 1989).

Statistical methods. Adjusted geometric mean plasma homocysteine was obtained by covariance analysis (Altman 1991) with the logarithm of total plasma homocysteine as the dependent variable. The model included five strong predictors of plasma homocysteine in this population and included gender, age (years), cigarette smoking (5 categories), coffee consumption (5 categories) and intake of vitamin supplements (5 categories).

The ability of gender and intake of vitamin supplements to predict hyperhomocysteinemia or hypohomocysteinemia was studied by multiple logistic regression (Altman 1991). Total plasma homocysteine was the dependent variable, whereas the independent variables were represented in the models as indicator variables denoting membership to one of two categories for gender and age and one of five categories for cigarette smoking, coffee consumption and intake of vitamin supplements. The OR estimated the risk that a man or a subject with a defined intake of vitamin supplements had a homocysteine level above or below a given threshold relative to a women or a subject not taking vitamins.

The analyses were performed with the statistical packages BMDP (Statistical Sciences 1993) and S-Plus (Dixon et al. 1988).

Opposite tails odds ratio profile. Opposite tails odds ratios (OTOR) include two values; the first is the OR for a homocysteine level above a defined percentile of the right tail, whereas the second is the inverse OR for a homocysteine level below the same percentile in the opposite (left) tail. Thus, an $OTOR_{95\text{-}5}$ of 4.4–2.5 for factor X indicates that the OTOR are calculated using the 95 and 5 percentiles as threshold levels. Compared with a subject without X, an individual with X has a relative risk of hyperhomocysteinemia of 4.4, whereas the relative risk of hypohomocysteinemia is 1/2.5, i.e., 0.4.

The OTOR profile involves a comparison between the two OR values and reflects the change in the homocysteine frequency distribution in the presence of an independent variable. In contrast to the OTOR values, which depend on the selected threshold levels, the profile is a characteristic trait of the factor investigated.

The concept of the OTOR profile is illustrated in **Figure 1.** Both factors Y and Z are known to increase the plasma homocysteine level. First, we select threshold levels for hypo- and hyperhomocysteinemia, for instance the 5 percentile (5 μmol/l) and 95 percentile (15 μmol/l) of the study population. We then categorize the population into those with and those without the factor. In Figure 1, the frequency distribution curve for subjects with Y shows a complete shift to the right compared with subjects without Y, and the $OR_{>95\%}$ is identical to $1/OR_{<5\%}$. This is referred to as a balanced OTOR profile. A different situation arises when sub-

[3] Abbreviations used: OR, odds ratio; OTOR, opposite tails odds ratios.

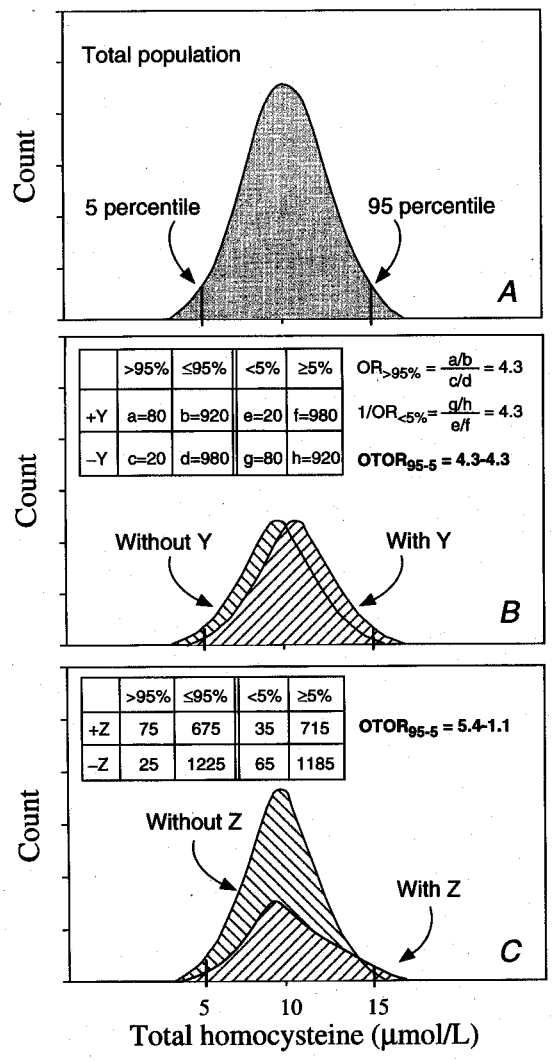

FIGURE 1 Examples of opposite tails odds ratios (OTOR) profiles when the presence of an independent variable is associated with a complete shift (factor Y) or selective upper shift (factor Z) in the frequency distribution of homocysteine. (***A***) The 5 and 95 percentile in a population (n = 2000) is 5 and 15 µmol/l, respectively. By categorizing the subjects into those with and without factor Y (***B***) or factor Z (***C***), two frequency distribution curves are obtained, and the odds ratio for hyperhomocysteinemia and hypohomocysteinemia can be calculated. The $OTOR_{95\text{-}5}$ refers to the obtained set of values of $OR_{>95\%}$ and $1/OR_{<5\%}$, i.e., 4.3–4.3 for factor Y and 5.4–1.1 for factor Z. For factor Y, these values are identical, i.e., the OTOR profile is balanced, indicating that Y induces a complete shift in the frequency distribution toward higher homocysteine levels. In contrast, there is an imbalance in the $OTOR_{95\text{-}5}$-values for factor Z, which suggests that this factor is associated with a selective shift of the upper tail.

jects with a factor Z have a different homocysteine distribution than subjects without factor Z (Fig. 1). Under these circumstances, the $OR_{>95\%}$ and $1/OR_{<5\%}$ are markedly different, i.e., the OTOR profile is imbalanced.

The interpretation of the OTOR profile can be summarized as follows: A balanced OTOR profile, i.e., both values are of similar magnitude above or below 1, suggests that the investigated factor is associated with complete shift of the frequency distribution curve. When the values are higher than 1, the factor induces a shift of the curve to the right, whereas values below 1 indicate a shift of the curve to the left.

An imbalanced OTOR profile usually indicates that the factor investigated is associated with a selective or predominant shift of one tail. Thus, an OR for hyperhomocysteinemia, which is substantially different from 1 combined with an 1/OR for hypohomocysteinemia close to 1, is referred to as a selective upper shift. In contrast, a selective lower shift is characterized by an OR for hyperhomocysteinemia close to 1, whereas the 1/OR for hypohomocysteinemia is substantially different from 1. An imbalanced OTOR profile, but with both values significantly different from 1, is described as a predominant upper or lower shift.

A third alternative is an imbalanced profile with one value above and the other below 1. This indicates that the factor is associated with a more or less symmetric compression or expansion of the frequency distribution curve.

RESULTS AND DISCUSSION

Adjusted mean plasma homocysteine was 10.92 (95% CI, 10.84–11.01) µmol/l and 9.61 (95% CI, 9.54–9.68) µmol/l for males and females, respectively. There was a dose-dependent decline in plasma homocysteine with increasing intake of vitamin supplements. In subjects with daily intake of vitamin supplements during the whole year, adjusted mean plasma homocysteine was 9.34 (95% CI, 9.2–9.49) µmol/l, compared with 10.55 (95% CI, 10.46–10.65) µmol/l in individuals who never took vitamin supplements. Thus, our data confirm previous studies (Ueland et al. 1992, Selhub et al. 1993) that females and subjects with high vitamin intake have lower plasma homocysteine levels than males and subjects with lower vitamin intake.

Using multiple logistic regression analyses, we investigated whether gender and intake of vitamin supplement exhibited a changed relation to plasma homocysteine in the extremes of the plasma homocysteine distribution. The top and bottom 1 (28.5 and 5.5 µmol/l), 2.5 (19.7 and 6.1 µmol/l), 5 (16.7 and 6.5 µmol/l) and 10 percentiles (14.3 and 7.1 µmol/l) of plasma homocysteine in the total population were selected as threshold levels and the OTOR calculated as shown in Figure 1.

For male sex relative to female sex, the OTOR profiles were markedly imbalanced, with $OTOR_{99\text{-}1}$ of 1.1–14.5 (**Table 1**). Thus, at the top and bottom percentile of a population with equal numbers of men and women, the predictive value of gender would be highly different. In the 1% of subjects with the lowest plasma homocysteine level, 94% would be women. In contrast, in the 1% of subjects with highest homocysteine level

TABLE 1

Describing the relation between plasma homocysteine and gender or intake of vitamin supplements by using opposite tails odds ratios (OTOR)[1]

	Percentiles of the homocysteine frequency distribution			
	99–1	97.5–2.5	95–5	90–10
	OTOR			
Male (vs. female)	1.08*–14.50	1.14*–11.30	1.30–6.95	1.52–5.44
Categories of vitamin supplement (vs. category 1)				
2	0.88*–0.48*	0.82*–0.72*	0.80*–0.78*	0.83–0.81
3	0.27–0.25	0.47–0.54	0.69–0.60	0.70–0.64
4	0.47–0.20	0.42–0.38	0.56–0.41	0.61–0.52
5	0.15–0.14	0.31–0.26	0.35–0.32	0.45–0.37

[1] The top and bottom 1, 2.5, 5 and 10 percentiles of the homocysteine frequency distribution for the total population were used as cut points for calculating the odds ratios for hyper- and hypohomocysteinemia. The results, expressed as OTOR, include adjustment for age, cigarette smoking, coffee consumption and intake of vitamin supplements or gender. The marked imbalance in the OTOR profiles for gender suggest a predominant or even selective shift of the lower tail towards higher homocysteine levels in men compared with women. The balanced OTOR profiles for intake of vitamin supplements indicate a complete shift of the homocysteine distribution to the left with increasing vitamin intake.
* Not significantly different from 1.

about 48% would be women and 52% men. Thus, the influence of gender on the homocysteine distribution represents an example of selective lower shift.

These results may explain the absence of a relation between homocysteine and gender in some previous investigations (Andersson et al. 1992, Kang et al. 1986). It may also have important clinical implications. Some studies have demonstrated a progressive reduction in vascular disease with declining plasma homocysteine level (Arnesen et al. 1995, Selhub et al. 1995). If plasma homocysteine is causally related to the atherosclerotic process, these results indicate that female sex is a protective factor due to the high frequency of women with low plasma homocysteine level. In contrast, if the harmful effect of homocysteine only becomes apparent at high levels, as suggested in the Physicians' Health study (Stampfer et al. 1992, Verhoef et al. 1994), the gender-induced difference in homocysteine probably represents only a small change in risk for vascular disease.

We found that intake of vitamin supplements every day during the whole year was associated with a change in mean plasma homocysteine level comparable with that observed between men and women, i.e., about 1.3 μmol/l. However, in contrast to gender, the OTOR profiles for intake of vitamin supplements were balanced (Table 1), suggesting a complete shift of the curve to the left. Although the influence of B-vitamins on homocysteine metabolism is well recognized (Allen et al. 1994, Selhub et al. 1993, Ubbink et al. 1993), the explanation for the sex difference is not clear but may be related vitamin status (Andersson et al. 1992, Selhub et al. 1993), muscle mass and hormonal factors (Ueland et al. 1992). In this population, the marked difference in the OTOR profiles for gender and intake of vitamin supplements suggest that these two factors are related to the homocysteine level through different mechanisms.

In conclusion, the relation between an independent variable and plasma homocysteine may vary at high and low homocysteine concentration. This may have important implications because strong relations may be missed because of inadequate statistical analyses of the data. Different homocysteine distribution, in shape or location, may also explain conflicting results from subgroup analyses, indicating the presence of relations in one group that is not found in another. As demonstrated for gender in the present study, the most pronounced relation may be observed in the low range of the homocysteine distribution. Finally, factors that are related to plasma homocysteine through the same mechanism would be expected to have a similar OTOR profile.

Note added in proof. *After this manuscript was accepted for publication, an official nomenclature committee (during the International Conference on Homocysteine Metabolism, Dromoland Castle, Ireland, July 2–6, 1995) recommended the use of the following abbreviation for total homocysteine: (tHcy).*

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