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Evaluation of the Metabotype Concept Identified in an Irish Population in the German KORA Cohort Study

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Scope: Previous work identified three metabolically homogeneous subgroups of individuals ("metabotypes") using *k*-means cluster analysis based on fasting serum levels of triacylglycerol, total cholesterol, HDL cholesterol, and glucose. The aim is to reproduce these findings and describe metabotype groups by dietary habits and by incident disease occurrence. Methods and results: 1744 participants from the KORA F4 study and 2221 participants from the KORA FF4 study are assigned to the three metabotype clusters previously identified by minimizing the Euclidean distances. In both KORA studies, the assignment of participants results in three metabolically distinct clusters, with cluster 3 representing the group of participants with the most unfavorable metabolic characteristics. Individuals of cluster 3 are further characterized by the highest incident disease occurrence during follow-up; they also reveal the most unfavorable diet with significantly lowest intakes of vegetables, dairy products, and fibers, and highest intakes of total, red, and processed meat. Conclusion: The three metabotypes originally identified in an Irish population are successfully reproduced. In addition to this validation approach, the observed differences in disease incidence across metabotypes represent an important new finding that strongly supports the metabotyping approach as a tool for risk stratification.

1. Introduction

The metabotyping concept is defined as the formation of metabolically/phenotypically homogeneous subgroups of individuals, so-called metabotypes or metabolic phenotypes.[1–6] This concept has been successfully applied in a number of different populations.[1,7,8] Due to its broad definition, studies showed large heterogeneities in its application, especially in the amount and type of metabolic parameters used for the identification of metabotypes.[7]

There are some studies that identified comprehensive metabotypes by the use of a large number of metabolic variables of different metabolic pathways, representing a detailed metabolic characterization of individuals.[8–14] For example, we used in previous projects various biochemical parameters from blood and urine as well as anthropometric measures for the identification of metabotypes in the German population-based

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Cooperative Health Research in the Region of Augsburg (KORA) cohort study.[8,15]

However, this comprehensive metabotyping approach is difficult to implement in the general population, as most of these metabolic parameters are not routinely measured in primary care. Thus, a reduced set of standard clinical parameters allowing the identification of distinct metabolic subgroups may be useful in practice, for example, for effective targeted strategies for disease prevention at the metabotype subgroup level.[1,7,16–19] In previous work in an Irish cross-sectional study in adults ($n = 875$), named Irish National Adult Nutrition Survey (NANS), O'Donovan et al.[20] identified three metabolically distinct subgroups of individuals using *k*-means cluster analysis based on the four clinical standard serum parameters triacylglycerol (TAG), total cholesterol (TC), HDL cholesterol, and glucose. This indicates that a small number of parameters seems to be sufficient to identify significant metabotypes.

The objective of the present study was to examine i) the reproducibility of this metabotyping concept, ii) the occurrence of incident diseases across metabotypes, and iii) the identification of significant differences in specific dietary habits between metabotype groups. Therefore, we assigned participants of the German population-based KORA cohort study to these three metabotype clusters, and characterized these metabotype clusters in detail.

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2. Experimental Section

2.1. Study Population

Analyses were performed on data from the population-based KORA F4 (2006–2008) and KORA FF4 (2013/2014) studies, which are the first and second follow-up examinations of the KORA S4 health survey conducted in the region of Augsburg in Southern Germany between 1999 and 2001.[21] In brief, of the 4261 participants aged 25–74 years included in the KORA S4 health survey, 3080 individuals and 2279 individuals also participated in the 7-year follow-up KORA F4 study and the 14-year follow-up KORA FF4 study, respectively. Of these, 2161 individuals participated in both follow-up studies. Information on the participation response has been described in detail elsewhere.[22] The participants of all studies were invited to the study center for a standardized physical examination and a computer-assisted personal interview, both conducted by trained staff. In addition, all participants answered self-administered questionnaires. Detailed information on these investigations, which were all conducted in accordance with the Declaration of Helsinki, has been provided previously.[23] All participants gave their written informed consent and the studies were approved by the Ethics Committee of the Bavarian Chamber of Physicians. To ensure comparability with previous investigations on metabotyping in the KORA studies,[8,15] the same sample sizes were used (KORA F4 study: $n = 1768$, KORA FF4 study: $n = 2279$).

2.2. Assessment of Biochemical Parameters for Metabotype Assignment and Characterization

Biochemical parameters were assessed in both KORA studies, KORA F4 and KORA FF4, using standard methods described previously.[8,24]

2.3. Assessment of Demographic, Anthropometric and Lifestyle Data for Metabotype Characterization

Demographic and lifestyle data were assessed in standardized face-to-face computer-assisted interviews and via self-administered questionnaires in the KORA F4 and KORA FF4 studies. These data included sex, age (in years), education (<10 years, 10 to < 12 years, ≥12 years), physical activity (active in both seasons summer and winter and active for ≥1 h per week in at least one season, inactive), and smoking status (non-smoker, ex-smoker, smoker). BMI was considered both continuously in kg m^{-2} and categorized into underweight (BMI < 18.5 kg m^{-2}), normal weight (BMI 18.5 kg m^{-2} to < 25 kg m^{-2}), overweight (BMI 25 kg m^{-2} to < 30 kg m^{-2}), and obese (BMI ≥ 30 kg m^{-2}).

2.4. Assessment of Cardiometabolic Disease for Metabotype Characterization

The presence of the following cardiometabolic diseases (yes/no) was assessed in the standardized face-to-face computer-assisted interviews and in the physical examinations in both studies, KORA F4 and KORA FF4. Type 2 diabetes was defined by either present intake of antidiabetic medication or a self-reported diagnosis, both validated with the respective treating physician. Hyperuricemia/gout and dyslipidemia were both determined by the self-reported current intake of the respective disease-specific medication. Previous inpatient treatment of myocardial infarction and stroke as well as cancer were also assessed by self-report. Hypertension was defined by the participants' awareness of a drug-controlled hypertension or by a blood pressure of ≥140/90 mmHg in the physical examinations.

For the determination of disease occurrence, all of these diseases were analyzed individually and combined into metabolic diseases (defined as suffering from at least one of the four metabolic diseases hypertension, type 2 diabetes, hyperuricemia/gout and dyslipidemia) and cardiovascular diseases (defined as inpatient treatment due to at least one of the two cardiovascular diseases myocardial infarction and stroke). In the KORA F4 study population, incident cases of diseases during the following 7 years were identified by means of disease occurrence in the KORA FF4 study in participants who did not suffer from the respective disease in the KORA F4 study.

2.5. Assessment of Dietary Intake for Metabotype Characterization

Dietary intake was assessed in the KORA FF4 study only. In total, 1602 KORA FF4 participants completed up to three 24 h food lists[25] and a food frequency questionnaire.[26] The usual dietary intake was estimated in an advanced blended two-step approach by combining the information of both dietary assessment instruments. This approach follows the idea of the National Cancer Institute (NCI) method[27] and the multiple source method[28] to initially separate the calculation of consumption probability and consumption amount on consumption days with regression models both including the same covariates to later connect the two parts. Subsequently, the usual dietary intake of all food items was computed for each participant by multiplying the consumption probability of a certain food item by the usual consumption amount on a consumption day. The food groups and subgroups were classified according to the European Prospective Investigation into Cancer and Nutrition (EPIC)-Soft classification system[29] and nutrients were determined using the National Nutrient Database (Bundeslebensmittelschlüssel BLS 3.02).

In addition to energy intake given in kJ d^{-1}, intakes of food groups, food subgroups and nutrients were specified in g d^{-1}: potatoes, vegetables, fruits, total dairy, milk, yoghurt, cheese, grains, total meat, red meat (beef and pork), poultry, processed meat, fish, eggs, fruit and vegetable juice, sugar-sweetened beverages, coffee, alcohol, total fiber, insoluble fiber, and soluble fiber.

2.6. Statistical Analysis

All statistical analyses were performed using the statistical software package RStudio version 1.0.136 that uses R version 3.2.2 (R Development Core Team, 2010, http://www.r-project.org). Statistical significance was determined as a $p < 0.05$.

2.6.1. Assignment of the KORA Participants to the Metabotype Clusters Identified in NANS

Of the selected samples of 1768 participants in the KORA F4 study and 2279 participants in the KORA FF4 study, excluded were those who were not fasting for at least 8 hours and those with missing data in the four grouping variables TAG, TC, HDL cholesterol, and glucose (KORA F4 study: $n = 24$; KORA FF4 study: $n = 58$) prior to cluster allocation. This resulted in final sample sizes of 1744 participants in the KORA F4 study and 2221 participants in the KORA FF4 study. These were respectively assigned to the metabotype cluster previously identified in NANS with the smallest total Euclidean distance of the four z-standardized values for TAG, TC, HDL cholesterol, and glucose to the respective z-standardized cluster centers (means) of these variables. The cluster centers are provided in Table S1, Supporting Information.

2.6.2. Descriptive Statistics of the Identified Metabotype Clusters in KORA

The metabotype clusters were described by medians, 25th and 75th percentiles of continuous variables and by total and relative frequencies of categorical variables. Statistically significant differences in these variables across metabotype clusters were determined by Kruskal–Wallis test for continuous variables and by Pearson's chi-squared test for categorical variables, which were followed by the respective post hoc tests with Bonferroni correction. All data are shown for the total study population and the three metabotype clusters, respectively. Due to missing values in descriptive variables, the respective maximum number of participants available was used for the calculations leading to different sample sizes between variables.

Table 1. Demographic characteristics of the total KORA F4 study population and across the three metabotype clusters.

	Total	Metabotypes			*p*-value
		Cluster 1	Cluster 2	Cluster 3	
	N = 1744	*N* = 590	*N* = 813	*N* = 341	
Sex					
Men	846 (48.5)	162 (27.5)	477 (58.7)	207 (60.7)	**<0.0001**
Women	898 (51.5)	428 (72.5)	336 (41.3)	134 (39.3)	—
Age [years]					
Median (25th, 75th)	61.0 (54.0, 68.0)	60.0 (53.0, 67.0)	60.0 (52.0, 68.0)	62.0 (55.0, 69.0)	**0.02**
Education [years]					
<10	175 (10.1)	59 (10.0)	84 (10.4)	32 (9.4)	**0.04**
10 to < 12	912 (52.4)	328 (55.6)	392 (48.4)	192 (56.3)	—
≥12	654 (37.6)	203 (34.4)	334 (41.2)	117 (34.3)	—
BMI [kg m^{-2}]					
Median (25th, 75th)	27.5 (24.8, 30.7)	25.7 (23.5, 28.7)	27.9 (25.2, 31.0)	29.7 (27.0, 32.7)	**<0.0001**
Underweight	1 (0.1)	0 (0.0)	1 (0.1)	0 (0.0)	**<0.0001**
Normal	461 (26.5)	240 (40.7)	188 (23.2)	33 (9.7)	—
Overweight	747 (43.0)	249 (42.2)	352 (43.5)	146 (43.1)	—
Obese	529 (30.4)	101 (17.1)	268 (33.1)	160 (47.2)	—
Physical activity					
Inactive	738 (42.3)	209 (35.4)	358 (44.1)	171 (50.1)	**<0.0001**
Active	1005 (57.7)	381 (64.6)	454 (55.9)	170 (49.9)	—
Smoking status					
Non-smoker	771 (44.2)	296 (50.2)	344 (42.4)	131 (38.4)	**0.0004**
Ex-smoker	718 (41.2)	226 (38.3)	350 (43.1)	142 (41.6)	—
Smoker	254 (14.6)	68 (11.5)	118 (14.5)	68 (19.9)	—

Median (25th, 75th percentile) for continuous variables and *n* (column %) for categorical variables; Kruskal–Wallis test for continuous variables and Pearson's chi-squared test for categorical variables; Significant results ($p < 0.05$) are highlighted in bold; Due to missing information, reduced datasets for education $n = 1741$, BMI $n = 1738$, physical activity $n = 1743$, and smoking status $n = 1743$; KORA, Cooperative Health Research in the Region of Augsburg.

3. Results

Table 1 shows the demographic characteristics of the total study population and of each of the three metabotype clusters identified in the KORA F4 study. The total study population aged 32–77 years comprised nearly equal proportions of men and women. Of 1744 participants, 590 (33.8%) were assigned to cluster 1, 813 (46.6%) to cluster 2 and 341 (19.6%) to cluster 3 by minimizing the total Euclidean distance of the four grouping variables to their cluster centers identified in NANS. The proportion of men was higher in clusters 2 (58.7%) and 3 (60.7%) compared to cluster 1 (27.5%). Cluster 3 was characterized as the cluster with the highest median age of 62.0 years (range = 45–77 years) and BMI of 29.7 kg m^{-2} (range = 21.5–47.6 kg m^{-2}) as well as the highest proportions of physically inactive individuals (50.1%) and smokers (19.9%). The proportion of highly educated individuals was highest in cluster 2 with 41.2%. Similar results were found in the KORA FF4 study and are displayed in Table S2, Supporting Information.

Table 2 presents the variation of the four grouping variables (TAG, TC, HDL cholesterol, and glucose) across the three metabotype clusters in the KORA F4 study population. Individuals in cluster 3 were characterized by the highest median values of TAG, TC and glucose as well as by the lowest median value of HDL cholesterol. Cluster 2 was comprised of individuals with the lowest TC levels and individuals in cluster 1 were found to have the highest HDL cholesterol levels and simultaneously the lowest levels of TAG and glucose. The same results were found in the KORA FF4 study population and are shown in Table S3, Supporting Information.

Table 3 displays the median values of further biochemical parameters of the total KORA F4 study population and across the three clusters. For most of the parameters, individuals in cluster 3 showed the highest and individuals in cluster 1 simultaneously the lowest median values. Cluster 2 was characterized by the significantly lower median values of LDL cholesterol, non-esterified fatty acids, aldosterone, and insulin-like-growth-factor-binding-protein-3 compared to clusters 1 and 3. Using the conservative Bonferroni method to correct for multiple testing, those results with $p < 0.0017$ still remain statistically significant. Table S4, Supporting Information, shows similar results for the KORA FF4 study population for the subset of biochemical parameters available in this study.

Table 4 presents the prevalence and incidence of cardiometabolic diseases across the three clusters in the KORA F4 study population. Cluster 3 showed the highest prevalence of hypertension, type 2 diabetes, hyperuricemia/gout and presence of any metabolic disease. The highest prevalence of

Table 2. Variation of the grouping variables across the three metabotype clusters of the KORA F4 study.

	Metabotypes			*p*-value
	Cluster 1 ($N = 590$)	Cluster 2 ($N = 813$)	Cluster 3 ($N = 341$)	
Grouping variables (serum fasting levels in mmol L^{-1})	Median (25%, 75%)	Median (25%, 75%)	Median (25%, 75%)	
TAG	**0.98** (0.73, 1.28)	1.21 (0.89, 1.60)	2.59 (2.07, 3.18)	**<0.0001**
TC	6.18 (5.66, 6.77)	**5.09** (4.60, 5.58)	6.33 (5.79, 6.98)	**<0.0001**
HDL cholesterol	1.81 (1.65, 2.02)	1.29 (1.14, 1.45)	**1.16** (1.03, 1.34)	**<0.0001**
Glucose	**5.11** (4.83, 5.50)	5.39 (5.00, 5.89)	5.89 (5.39, 6.72)	**<0.0001**

Median (25th, 75th percentile); Kruskal–Wallis test; Significant results ($p < 0.05$) are highlighted in bold; Underlined values represent the highest value across the clusters; the bold values represent the lowest; KORA, Cooperative Health Research in the Region of Augsburg; TAG, triacylglycerol; TC, total cholesterol.

dyslipidemia, myocardial infarction and of any cardiovascular disease was found in cluster 2, closely followed by cluster 3. The incidence of all individual metabolic diseases as well as of any metabolic or any cardiovascular disease was highest in cluster 3. Comparable results of cardiometabolic disease prevalence in the KORA FF4 study population are shown in Table S5, Supporting Information.

As the usual dietary intake was not assessed in the KORA F4 study but in the KORA FF4 study, **Table 5** presents the median usual dietary intake of the total KORA FF4 study population and across the three metabotype clusters identified in this study. The participants assigned to cluster 3 showed significantly higher consumption of total meat, red meat, and processed meat and significantly lower intake of vegetables, total dairy, milk, yoghurt, total fiber, insoluble fiber, and soluble fiber compared to clusters 1 and 2. In contrast, participants of cluster 1 consumed the highest amounts of vegetables and yoghurt and the lowest amounts of grains, total meat, red meat, poultry, processed meat, fruit and vegetable juice, sugar-sweetened beverages and total energy. Participants grouped into cluster 2 showed mainly intermediate median intake amounts. After applying the conservative Bonferroni method to correct for multiple testing, those results with $p < 0.0023$ still remain statistically significant.

4. Discussion

Using previously published metabotypes,[20] we identified three metabolically distinct clusters of individuals in each of the two KORA studies. Cluster 3 represented the group of participants with the most unfavorable metabolic characteristics. Individuals of cluster 3 were further characterized by the highest disease occurrence and the most unfavorable diet. Thus, we could successfully reproduce the metabotype concept based on a minimal number of biochemical parameters, a prerequisite for further development of such a concept. The shown differences in the occurrence of incident diseases by metabotype group clearly demonstrate that metabotyping can be used as a tool for the identification of high-risk groups. In combination with the observed differences in habitual dietary characteristics, the metabotype concept is still very likely to approach our expectation, that is, the development of a practical tool for personalized prevention of cardio-metabolic diseases.

4.1. Comparison of Findings with the Originally Identified Metabotypes in NANS

Regarding the metabolic parameters (TAG, TC, HDL cholesterol, and glucose) measured in all three studies, NANS and the KORA F4/FF4 studies, it is interesting to note that the values are more favorable in NANS than in the KORA studies across all three metabotype clusters.[20] This suggests that the NANS study population was generally healthier than the KORA F4 and KORA FF4 study populations, which is supported by the high disease prevalence and incidence in the KORA studies. Furthermore, the NANS population was markedly younger compared to the KORA F4/FF4 populations (median age of 41 vs 61/60 years) and it is well accepted that the physiological aging process is associated with adverse alterations in metabolism and metabolic flexibility.[30] Besides age, the human metabolism is further influenced by a variety of intrinsic and environmental factors such as genetics, epigenetics, gut microbiome or body composition in conjunction with lifestyle factors such as physical activity and diet.[16,17,31,32] Despite these considerations, we demonstrated that metabotypes established in one population group could be used to classify individuals in another group. Overall, our results clearly indicate that we have successfully used the metabotypes identified by O'Donovan et al.[20] in the Irish NANS by classifying individuals of the German KORA F4 and KORA FF4 study populations into metabolically distinct subgroups. Our work suggests that the metabotype concept is transferrable and applicable to other ethnically similar populations.

4.2. Comparison of Findings with Previously Identified Metabotypes in KORA

In previous analyses on metabotyping,[8,15] we also identified three metabotype clusters in both the KORA F4 and the KORA FF4 studies using extensive sets of grouping variables. We used in total 34 parameters in the KORA F4 study and a subset of 16 parameters in the KORA FF4 study.

In both metabotyping approaches used in the KORA studies, that is, assignment of participants to existing metabotype clusters identified in NANS (conducted here) and identification of metabotype clusters by cluster analysis,[8,15] a more metabolically unfavorable cluster 3, an intermediate cluster 2 and a more

Table 3. Median values of biochemical parameters of the total study population and across the three metabotype clusters, KORA F4 study.

	Total	Metabotypes			*p*-value
		Cluster 1	Cluster 2	Cluster 3	
	N = 1744	*N* = 590	*N* = 813	*N* = 341	
TC/HDL cholesterol	3.98 (3.31, 4.78)	**3.37** (2.91, 3.93)[a]	3.98 (3.44, 4.61)[b]	5.40 (4.73, 6.12)[c]	**<0.0001**
Glycated hemoglobin [%]	5.5 (5.3, 5.8)	5.5 (5.2, 5.6)[a]	5.5 (5.3, 5.8)[b]	5.7 (5.4, 6.1)[c]	**<0.0001**
Uric acid [μmol L^{-1}]	313 (255, 375)	**270** (229, 327)[a]	318 (268, 378)[b]	365 (313, 426)[c]	**<0.0001**
LDL cholesterol [mmol L^{-1}]	3.57 (3.00, 4.19)	3.80 (3.19, 4.44)[a]	**3.28** (2.76, 3.75)[b]	4.11 (3.44, 4.68)[c]	**<0.0001**
Leukocytes [nL^{-1}]	5.7 (4.8, 6.7)	**5.3** (4.5, 6.3)[a]	5.7 (4.9, 6.8)[b]	6.1 (5.1, 7.3)[c]	**<0.0001**
Glutamate–pyruvate transaminase [μkat L^{-1}]	0.37 (0.27, 0.51)	**0.32** (0.26, 0.42)[a]	0.38 (0.27, 0.53)[b]	0.45 (0.34, 0.67)[c]	**<0.0001**
Glutamate–oxaloacetate transaminase [μkat L^{-1}]	0.42 (0.36, 0.50)	**0.40** (0.35, 0.48)[a]	0.43 (0.35, 0.50)[a]	0.44 (0.37, 0.54)[b]	**<0.0001**
Gamma-glutamyltransferase [μkat L^{-1}]	0.47 (0.31, 0.76)	**0.38** (0.27, 0.62)[a]	0.46 (0.31, 0.70)[b]	0.67 (0.47, 1.04)[c]	**<0.0001**
Alkaline phosphatase [μkat L^{-1}]	1.13 (0.95, 1.36)	1.13 (0.92, 1.34)[a]	**1.11** (0.95, 1.36)[a]	1.20 (1.02, 1.39)[b]	**0.001**
Average telomere length in leukocytes (ratio of the telomere repeat copy number to a single copy gene)	1.77 (1.59, 1.98)	1.80 (1.61, 2.00)[a]	1.76 (1.59, 1.98)[a,b]	**1.75** (1.57, 1.95)[b]	**0.02**
Non-esterified fatty acids [mg dL^{-1}]	6.92 (5.34, 8.74)	7.04 (5.54, 8.93)[a]	**6.60** (4.94, 8.44)[b]	7.49 (5.87, 9.13)[a]	**<0.0001**
Lipoprotein(a) [mg dL^{-1}]	12.2 (5.5, 31.9)	14.0 (6.1, 34.8)[a]	**11.5** (4.9, 28.6)[b]	12.3 (5.6, 32.7)[a,b]	**0.02**
Apolipoprotein A-IV [mg dL^{-1}]	15.1 (12.6, 17.9)	15.7 (12.8, 18.3)[a]	**14.9** (12.5, 17.5)[b]	15.2 (12.4, 18.2)[a,b]	**0.02**
Afamin [mg L^{-1}]	71.3 (61.2, 82.8)	**67.6** (59.1, 77.4)[a]	70.3 (60.3, 81.0)[b]	83.1 (71.4, 95.4)[c]	**<0.0001**
Leptin [ng mL^{-1}]	13.4 (6.2, 26.4)	14.0 (6.4, 24.7)[a]	**12.0** (5.5, 25.6)[a]	16.0 (7.8, 33.1)[b]	**<0.0001**
Thyroperoxidase antibodies [IU mL^{-1}]	12 (11, 15)	12 (11, 16)[a]	12 (11, 15)[a]	12 (11, 15)[a]	0.82
Cystatin C [mg L^{-1}]	0.74 (0.68, 0.83)	**0.72** (0.65, 0.79)[a]	0.75 (0.68, 0.83)[b]	0.78 (0.71, 0.87)[c]	**<0.0001**
High-sensitivity C-reactive protein [mg L^{-1}]	1.28 (0.63, 2.66)	**1.10** (0.54, 2.09)[a]	1.18 (0.61, 2.64)[b]	1.88 (0.98, 3.62)[c]	**<0.0001**
Urine albumin [mg L^{-1}]	7.5 (3.9, 16.8)	**6.6** (3.3, 13.5)[a]	7.7 (4.0, 17.0)[b]	11.3 (4.8, 23.0)[c]	**<0.0001**
Urine creatinine [g L^{-1}]	1.31 (0.84, 1.92)	**1.20** (0.74, 1.74)[a]	1.34 (0.87, 1.99)[b]	1.48 (0.96, 2.06)[b]	**<0.0001**
Interleukin-18 [pg mL^{-1}]	299 (232, 389)	**268** (205, 345)[a]	314 (239, 409)[b]	325 (262, 418)[b]	**<0.0001**
Insulin-like growth factor-1 [ng mL^{-1}]	126 (100, 155)	127 (102, 154)[a]	127 (100, 159)[a]	**119** (95, 147)[b]	**0.01**
Renin [μIU mL^{-1}]	10.8 (5.7, 19.0)	**9.9** (5.4, 16.8)[a]	10.8 (5.6, 20.4)[a]	12.4 (7.1, 24.0)[b]	**<0.0001**
Aldosterone [pg mL^{-1}]	38 (26, 56)	40 (28, 58)[a]	**36** (24, 54)[b]	40 (27, 59)[a]	**0.01**
Insulin-like-growth-factor-binding-protein-3 [ng mL^{-1}]	3390 (2835, 3970)	3400 (2910, 3960)[a]	**3275** (2700, 3830)[b]	3720 (3085, 4280)[c]	**<0.0001**
Sex-hormone-binding globulin [nmol L^{-1}]	30.5 (23.1, 42.2)	36.5 (27.7, 48.5)[a]	29.4 (22.7, 40.5)[b]	**24.5** (18.6, 33.4)[c]	**<0.0001**
Thyroid-stimulating hormone [mIU L^{-1}]	1.24 (0.81, 1.80)	1.22 (0.76, 1.77)[a]	1.22 (0.82, 1.79)[a]	1.33 (0.83, 1.90)[a]	0.16
Free thyroxine [pmol L^{-1}]	14.0 (12.7, 15.4)	13.9 (12.7, 15.3)[a,b]	14.1 (12.9, 15.8)[a]	**13.7** (12.4, 14.9)[b]	**0.002**
Insulin [μIU mL^{-1}]	4.4 (2.9, 7.9)	**3.4** (2.3, 5.0)[a]	4.6 (3.2, 8.7)[b]	6.8 (4.3, 14.4)[c]	**<0.0001**

Median (25th, 75th percentile); Kruskal–Wallis test (and Kruskal–Wallis post hoc test with Bonferroni correction); Significant results ($p < 0.05$) are highlighted in bold. Different superscript letters between clusters indicate a significant difference between clusters, whereas the same superscript letters between clusters indicate no significant difference between clusters; Underlined values represent the highest value across the clusters; the **bold** values represent the lowest; Due to missing values reduced data sets for leukocytes *n* = 1743, glutamate–pyruvate transaminase *n* = 1741, glutamate–oxaloacetate transaminase *n* = 1741, gamma-glutamyltransferase *n* = 1741, alkaline phosphatase *n* = 1741, average telomere length in leukocytes *n* = 1735, non-esterified fatty acids *n* = 1743, lipoprotein(a) *n* = 1743, apolipoprotein A-IV *n* = 1743, afamin *n* = 1743, leptin *n* = 1741, Thyroperoxidase antibodies *n* = 1691, cystatin C *n* = 1742, high-sensitivity C-reactive protein *n* = 1742, urine albumin *n* = 1736, urine creatinine *n* = 1735, interleukin-18 *n* = 1733, insulin-like growth factor-1 *n* = 1743, renin *n* = 1728, aldosterone *n* = 1731, insulin-like-growth-factor-binding-protein-3 *n* = 1727, sex-hormone-binding globulin *n* = 1726, thyroid-stimulating hormone *n* = 1704, free thyroxine *n* = 1711, and insulin *n* = 1742; KORA, Cooperative Health Research in the Region of Augsburg; TC, total cholesterol.

metabolically favorable cluster 1 were found. However, the two approaches resulted in a difference in the number of individuals per cluster for cluster 1 (34% vs 44%) and cluster 2 (46% vs 36%) with a stable percentage but not the identical individuals in cluster 3 (20% vs 20%). The median values of fasting blood lipids across clusters also varied between the metabotyping approaches. The most unfavorable fasting blood lipid values were seen in cluster 3 of the metabotype clusters identified by assignment to the NANS metabotype clusters, which is in contrast to the metabotype clusters identified by cluster analysis with the most unfavorable blood lipid values in the intermediate cluster 2. The disease prevalence and incidence was significantly different across metabotype clusters identified by cluster assignment, especially for type 2 diabetes and hyperuricemia/gout. Using cluster analysis based on extensive parameters, the disease occurrence was higher in cluster 3 and, thus, a stronger risk group was identified than by cluster assignment based on the four parameters. These differences were mainly evoked by the different sets of grouping variables. This means that individuals could be assigned easily and reasonably to existing metabotypes without grouping each population separately by cluster analysis, and this would be especially relevant in clinical practice.

Table 4. Disease prevalence and incidence in the total study population and across the three metabotype clusters, KORA F4/FF4 study.

	Total	Metabotypes			*p*-value
		Cluster 1	Cluster 2	Cluster 3	
	N = 1744	*N* = 590	*N* = 813	*N* = 341	
Prevalence of diseases in KORA F4		% [*n*]			
Hypertension	45.5 (793)	31.7 (187)	49.1 (399)	60.9 (207)	**<0.0001**
Type 2 diabetes	8.8 (153)	1.9 (11)	9.5 (77)	19.1 (65)	**<0.0001**
Hyperuricemia/gout	4.6 (81)	1.9 (11)	5.0 (41)	8.5 (29)	**<0.0001**
Dyslipidemia	16.6 (290)	9.8 (58)	21.2 (172)	17.6 (60)	**<0.0001**
Any of above metabolic diseases	52.4 (912)	36.3 (214)	57.4 (466)	68.4 (232)	**<0.0001**
Myocardial infarction	3.6 (62)	1.2 (7)	4.8 (39)	4.7 (16)	**0.001**
Stroke	2.7 (47)	1.7 (10)	3.6 (29)	2.3 (8)	0.09
Any of above cardiovascular diseases	5.9 (103)	2.7 (16)	7.8 (63)	7.0 (24)	**0.0002**
Cancer	9.1 (159)	7.3 (43)	10.0 (81)	10.3 (35)	0.16
Incidence of diseases in KORA FF4		% [*n*]			
Hypertension	22.8 (168)	18.0 (55)	25.9 (86)	27.0 (27)	**0.03**
Type 2 diabetes	6.7 (78)	2.8 (12)	7.0 (38)	14.9 (28)	**<0.0001**
Hyperuricemia/gout	3.0 (36)	1.2 (5)	3.6 (20)	5.3 (11)	**0.01**
Dyslipidemia	12.3 (132)	10.2 (41)	8.4 (40)	26.6 (51)	**<0.0001**
Any of above metabolic diseases	27.7 (180)	22.8 (65)	27.6 (78)	45.7 (37)	**0.0003**
Myocardial infarction	2.0 (24)	0.9 (4)	2.3 (13)	3.2 (7)	0.07
Stroke	2.4 (29)	1.4 (6)	2.4 (14)	4.1 (9)	0.10
Any of above cardiovascular diseases	4.1 (49)	2.1 (9)	4.6 (25)	7.0 (15)	**0.01**
Cancer	6.9 (79)	6.8 (28)	7.7 (41)	5.0 (10)	0.43

Column % (*n*); Pearson's chi-squared test (Fisher's exact test if expected frequencies were too low); Significant results ($p < 0.05$) are highlighted in bold; Prevalence: Due to missing information, reduced datasets for hypertension $n = 1741$, type 2 diabetes $n = 1743$, hyperuricemia/gout $n = 1743$, dyslipidemia $n = 1743$, all metabolic diseases $n = 1740$, myocardial infarction $n = 1743$, stroke $n = 1743$, all cardiovascular diseases $n = 1743$, and cancer $n = 1743$; Incidence: Due to missing information, reduced datasets for hypertension $n = 738$, type 2 diabetes $n = 1165$, hyperuricemia/gout $n = 1202$, dyslipidemia $n = 1069$, all metabolic diseases $n = 649$, myocardial infarction $n = 1213$, stroke $n = 1234$, all cardiovascular diseases $n = 1195$, and cancer $n = 1148$; KORA, Cooperative Health Research in the Region of Augsburg.

Further examinations should focus on the determination of a uniform set of grouping variables, which are routinely and easily measured in research and clinical practice, but simultaneously enable the identification of precise and metabolically significantly different metabotypes possibly with a strong risk group for diseases. This could be useful to improve the comparability and transferability of metabotypes across populations. In addition, it is planned that the metabotypes identified in the KORA cohort study by cluster analysis will be applied as well in NANS and other studies to test their applicability in other populations.

4.3. Metabotypes for the Development of Targeted Strategies

Metabotypes as described here may be useful for the development and establishment of targeted strategies at a group level.[1] For the metabotype clusters identified in NANS and also for metabotype clusters identified in the European Food4Me study, O'Donovan et al.[20,33] previously developed decision tree approaches for targeted dietary advice, which showed high accordance with personalized advice. Thus, metabotyping seems to be a promising tool to simplify the delivery of effective advice to large populations.[1,16–19] As unhealthy dietary behavior and physical inactivity are major risk factors for many cardiometabolic diseases,[34,35] targeted lifestyle approaches may be useful in disease prevention and treatment. This seems to be especially relevant for metabotype cluster 3 with the highest occurrence of diseases and risk factors. Individuals in cluster 3 could be generally adviced to reduce the relatively high consumption of meat and to increase the relatively low consumption of vegetables and physical activity compared to clusters 1 and 2. However, for the development of targeted disease prevention strategies, it is necessary to identify differences between metabotype clusters in their association of diet and/or physical activity with disease-specific outcomes. Since only few studies investigated such differences between metabotypes,[10,15,36–38] further studies are needed for the development of targeted disease prevention strategies on the metabotype subgroup level and for testing the effectiveness. In addition, this could be also relevant for more specific metabolically subgroups such as of diabetic patients.[39,40] In that case however, personalized disease treatment on the individual's level seems to be more effective than targeted strategies on the metabotype subgroup level.[41]

4.4. Strengths and Limitations

One strength of the study is that we successfully applied the metabotypes identified in NANS in a large population-based

Table 5. Usual dietary intake of the total study population and across the three metabotype clusters, KORA FF4 study.

	Total	Metabotypes			*p*-value
		Cluster 1	Cluster 2	Cluster 3	
	$N = 2221$	$N = 764$	$N = 1019$	$N = 438$	
Food groups or subgroups					
Potatoes [g d^{-1}]	56 (45, 71)	**54** (44, 68)[a]	56 (45, 73)[a,b]	58 (48, 76)[b]	**0.01**
Vegetables [g d^{-1}]	164 (133, 207)	174 (142, 216)[a]	162 (131, 203)[b]	**153** (124, 190)[c]	**<0.0001**
Fruits [g d^{-1}]	147 (91, 207)	153 (103, 208)[a]	145 (90, 208)[a,b]	**130** (80, 204)[b]	**0.01**
Total dairy [g d^{-1}]	178 (115, 257)	187 (129, 265)[a]	180 (113, 263)[a]	**160** (101, 218)[b]	**<0.0001**
Milk [g d^{-1}]	69 (24, 132)	77 (27, 136)[a]	70 (22, 140)[a]	**58** (22, 102)[b]	**0.003**
Yoghurt [g d^{-1}]	31 (14, 67)	35 (16, 73)[a]	30 (13, 67)[b]	**22** (12, 48)[c]	**<0.0001**
Cheese [g d^{-1}]	27 (19, 37)	28 (20, 37)[a]	27 (19, 38)[a,b]	**25** (18, 35)[b]	**0.03**
Grains [g d^{-1}]	162 (133, 195)	**152** (127, 184)[a]	169 (139, 206)[b]	164 (138, 191)[b]	**<0.0001**
Total meat [g d^{-1}]	107 (83, 142)	**90** (74, 113)[a]	116 (90, 149)[b]	128 (98, 157)[c]	**<0.0001**
Red meat [g d^{-1}]	26 (19, 34)	**21** (17, 29)[a]	28 (20, 37)[b]	30 (23, 38)[c]	**<0.0001**
Poultry [g d^{-1}]	13 (10, 18)	**11** (9, 17)[a]	13 (11, 18)[b]	13 (11, 19)[b]	**<0.0001**
Processed meat [g d^{-1}]	42 (30, 62)	**34** (24, 49)[a]	46 (32, 65)[b]	52 (38, 75)[c]	**<0.0001**
Fish [g d^{-1}]	17 (12, 25)	**16** (12, 25)[a]	17 (12, 25)[a]	17 (12, 26)[a]	0.17
Eggs [g d^{-1}]	11 (8, 17)	11 (8, 16)[a,b]	11 (8, 17)[a]	12 (8, 19)[b]	**0.04**
Fruit and vegetable juice [g d^{-1}]	42 (24, 117)	**35** (22, 100)[a]	45 (25, 130)[b]	45 (27, 136)[b]	**<0.0001**
Sugar-sweetened beverages [g d^{-1}]	6 (4, 20)	**4** (3, 8)[a]	8 (4, 38)[b]	9 (5, 50)[b]	**<0.0001**
Coffee [g d^{-1}]	435 (361, 480)	437 (363, 476)[a]	**434** (366, 484)[a]	437 (353, 482)[a]	0.95
Nutrients					
Energy [kJ d^{-1}]	7680 (6520, 8920)	**7172** (6368, 8516)[a]	7949 (6636, 9178)[b]	7895 (6656, 8892)[b]	**<0.0001**
Alcohol [g d^{-1}]	5 (3, 14)	5 (2, 13)[a]	5 (3, 15)[a]	6 (3, 19)[a]	**0.04**
Total fiber [g d^{-1}]	17 (14, 20)	18 (15, 21)[a]	17 (14, 20)[a]	**16** (14, 19)[b]	**<0.0001**
Insoluble fiber [g d^{-1}]	11 (10, 14)	12 (10, 14)[a]	12 (10, 14)[a]	**11** (9, 13)[b]	**<0.0001**
Soluble fiber [g d^{-1}]	6 (5, 7)	6 (5, 7)[a]	6 (5, 7)[a]	**5** (5, 6)[b]	**0.001**

Median (25th, 75th percentile); Kruskal–Wallis test (and Kruskal–Wallis post hoc test with Bonferroni correction); Significant results ($p < 0.05$) are highlighted in bold. Different superscript letters between clusters indicate a significant difference between clusters, whereas the same superscript letters between clusters indicate no significant difference between clusters; Underlined values represent the highest value across the clusters and the **bold** values represent the lowest; Due to missing information, reduced datasets for all dietary intake variables: total $n = 1562$, cluster 1 $n = 555$, cluster 2 $n = 715$, cluster 3 $n = 292$; KORA, Cooperative Health Research in the Region of Augsburg.

cohort by identifying metabolically distinct clusters of individuals. These results should be confirmed in further studies to allow the use of the metabotype concept across different populations. Due to the absence of a uniform metabotype definition so far, it may be also worth replicating metabotyping approaches of other studies in different cohorts and comparing the results. This could lead to a more general and consistent metabotype definition and classification. Another strength of the present work is the availability of extensive data for the characterization of metabotypes in the KORA studies. Dietary intake was collected in detail by food frequency questionnaire and by up to three 24 h food lists in the KORA FF4 study and those data are the basis for targeted dietary advice. In addition, the cardiometabolic disease status was assessed in both KORA studies, KORA F4 and KORA FF4, so that disease incidence could be determined during the relatively long follow-up of 7 years between both studies. However, despite the influence of antidiabetic and lipid-lowering medication intake on the grouping parameters, we did not exclude individuals with prevalent type 2 diabetes and/or dyslipidemia before cluster assignment to ensure sufficient sample sizes for a meanigful metabotype characterization. Another limitation of this work is that data on diet and disease status were mainly based on self-report, known to be prone to misreporting.

5. Conclusions

Our successful replication of this metabotype concept—based on four commonly measured clinical parameters only—in another European population is a prerequisite for further developing this approach. Using data on incident disease occurrence, we could demonstrate for the first time that this is a promising concept to identify high-risk groups in the population that would most benefit from prevention measures. Given that the groups are differential in their dietary habits, the idea is to develop a full model for personalized prevention with specific dietary modification. However, further replication and identification of differences in lifestyle-disease associations between metabotypes seems necessary for the development of targeted disease prevention strategies. Before developing a decision tree leading to differential recommendations depending on the subjects' metabotype, further work on the metabotyping concept itself is

necessary. A comparison of models based on a minimal number of parameters (as applied here), a comprehensive number of biochemical parameters,[8] and an in-between solution will be performed to clarify what is the best model in terms of risk prediction and practicability.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

A.R. conceived and conducted the data analyses, interpreted the data, and wrote the manuscript; E.H. and N.W. contributed to data analyses and interpretation, and revised the manuscript; C.M., A.P., M.R., F.K., C.H., W.R., H.V., M.R., W.K., and H.W. were involved in the study organization, provided data, and reviewed the manuscript; H.D. and H.H. contributed to data interpretation and revised the manuscript; L.B. and J.L. conceived and designed the data analyses, contributed to data analyses and interpretation, and revised the manuscript; all authors have read and approved the final manuscript.

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