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Cohort Profile

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Cohort Profile: The Nijmegen Biomedical Study (NBS)

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Why was the cohort set up?

The Nijmegen Biomedical Study (NBS) is a population-based study that was initiated (by L.K. and A.V.) in 2000 in the municipality of Nijmegen (∼ 150 000 inhabitants) in the eastern part of The Netherlands. The NBS was originally established to obtain a universal reference population to be used for studies of genetic variation, lifestyle and environmental exposures in relation to traits or diseases of interest. However, NBS has also proven useful for studying population traits. The NBS was set up by the Department for Health Evidence, the Department of Laboratory Medicine and the Department of Internal Medicine of the Radboud university medical center (Radboudumc) in Nijmegen in collaboration with the municipality of Nijmegen and the community health service of Nijmegen. At a later stage, the Department of Human Genetics of the Radboudumc joined the NBS project team.

Approval to conduct the NBS was obtained from the Radboud university medical center Institutional Review Board. All participants gave written informed consent.

Who is in the cohort?

The logistic set-up of the NBS was tested in a pilot study performed between November 2001 and February 2002. Via the population registers of the municipality of Nijmegen, the names and addresses of 650 males and females aged 18 years and older were obtained. They each received a questionnaire with questions about, for example, lifestyle and health status. Of the 650, 342 questionnaires were filled out and returned (response rate 53%). Of these 342 persons, 262 (77%) also donated a blood sample. Execution of the pilot study led to further optimization of the study procedure, especially in the blood sampling procedure: the number of community offices for blood donation was decreased and their opening hours were extended.

In 2002, the first phase of NBS (NBS-1) was started. On 1 July, a random sample from the register of the municipality of Nijmegen was drawn, stratified by sex and 5-year age groups. Eligibility criteria were age 18 years or older, not living in an institution or rest home and the ability to fill out a questionnaire in Dutch. In total, 22 451 inhabitants of the municipality of Nijmegen were invited to fill out a postal questionnaire (NBS-1 QN) and to donate an 8.5-ml blood sample in a serum separator tube and one (for $N \sim 5000$) or two (for $N \sim 1500$) 10-ml EDTA blood samples. Of the invited participants, 96% were of Dutch nationality. Table 1 shows the age distribution of the invited

Table 1. Age and sex distribution of the sample taken on 1 July 2002

	Males			Females		
Age group (years)	*N* invited (% of total)	*N* of QN returned (% of *N* invited)	*N* of blood samples donated (% of *N* invited)	*N* invited (% of total)	*N* of QN returned (% of *N* invited)	*N* of blood samples donated (% of *N* invited)
18-24	828 (8%)	247 (30%)	108 (13%)	826 (7%)	425 (52%)	215 (26%)
25-29	852 (8%)	230 (27%)	117 (14%)	864 (8%)	373 (43%)	195 (23%)
30-34	881 (8%)	268 (30%)	137 (16%)	866 (8%)	408 (47%)	227 (26%)
35-39	853 (8%)	262 (31%)	152 (18%)	837 (7%)	383 (46%)	256 (31%)
40-44	833 (8%)	297 (36%)	185 (22%)	803 (7%)	372 (46%)	258 (32%)
45-49	808 (7%)	336 (42%)	216 (27%)	799 (7%)	422 (53%)	307 (38%)
50-54	801 (7%)	325 (41%)	228 (29%)	802 (7%)	402 (50%)	311 (39%)
55-59	805 (7%)	346 (43%)	269 (33%)	788 (7%)	358 (45%)	298 (38%)
60-64	819 (7%)	384 (47%)	312 (38%)	787 (7%)	409 (52%)	333 (42%)
65-69	811 (7%)	433 (53%)	348 (43%)	786 (7%)	405 (52%)	323 (41%)
70-74	778 (7%)	391 (50%)	310 (40%)	766 (7%)	344 (45%)	267 (35%)
75-79	766 (7%)	366 (48%)	299 (39%)	762 (7%)	298 (39%)	214 (28%)
80-84	755 (7%)	307 (41%)	232 (31%)	750 (9%)	225 (30%)	146 (20%)
≥ 85	363 (3%)	115 (32%)	80 (22%)	1,062 (7%)	219 (21%)	125 (12%)
Total	10953 (100%)	4307 (39%)	2993 (27%)	11498 (100%)	5043 (44%)	3475 (30%)

participants, separately for males and females. Sampling fractions per sex and 5-year age group are available on request.

The overall response to the questionnaire was 42% (N = 9350), and 69% (N = 6468) of the responders donated blood samples. Table 1 shows an overview of response rates stratified by age and gender. The main reasons for non-participation based on a telephone survey among 65 non-responders were 'not interested' (32%), and 'age (too old)' (14%).

How often have they been followed up?

The first phase of NBS has been followed by four additional phases across the period 2002-16 (Figure 1). There are currently no plans to conduct additional rounds of follow-up.

NBS-2 and NBS-2-NIMA

NBS-2 was initiated in 2005, with additional health-related questions of interest to the researchers. All participants of NBS-1 who had given permission to be approached for further research were invited to participate. Based on their age on 1 November 2005, participants were invited for different additional methods of data collection, in addition to the basic NBS-2 questionnaire (NBS-2 QN), which was sent to all.

Age below 50 years

Of the 2926 participants who were approached to fill out the NBS-2 QN, 1884 (64%) completed and returned the QN.

Age between 50 and 70 years

Of the 2807 participants, 2114 (75%) filled out the NBS-2 QN. Furthermore, 1517 of the 2807 people (54%) participated in the NBS-2-NIMA1 study at the Department of Internal Medicine; this sub-study focused on cardiovascular risk prediction using non-invasive measurements of atherosclerosis (NIMA), namely intima-media thickness (IMT), endothelium function using flow-mediated dilatation (FMD), ankle-brachial index (ABI) and pulse-wave velocity (PWV). All NIMA1 participants (N = 1517) were re-invited in 2012 to fill out an additional questionnaire; 1423 (87%) responded. In addition, 95 of 100 randomly invited NIMA1 participants underwent repeated NIMA at this time. Of these 95 participants, 20 were measured again within 2 weeks to determine repeatability of the measurements. Currently NBS-2-NIMA3 is being executed, with focus on the role of intestinal bacteria in the development of atherosclerosis. Invitations were sent to participants of NBS-2-NIMA2 with a body mass index (BMI) > 27 kg/m^2 (N = 561) and to participants with a BMI > 25 kg/m^2 and < 27 kg/m^2 who indicated that their weight had increased (N = 397); also, friends/family of the participants (N = 145) were invited. Of all invited persons, 302 participated in this part of the study.

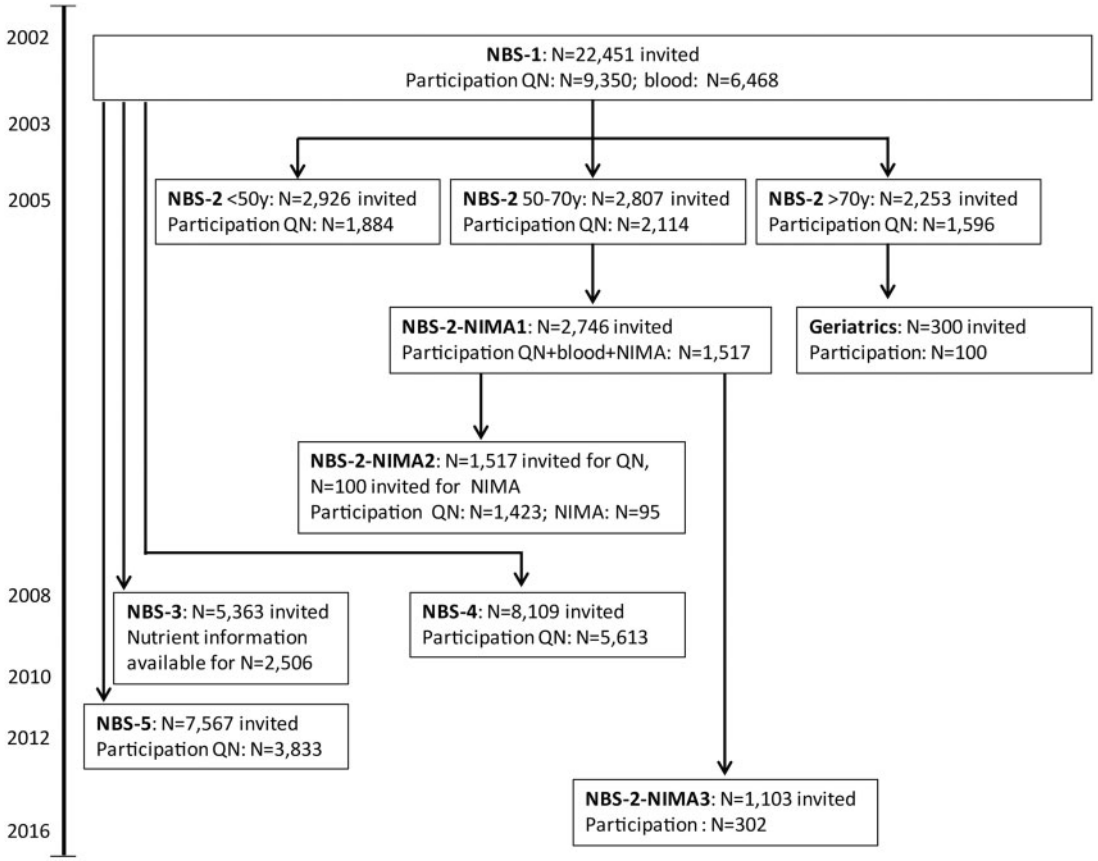

Figure 1. Schematic overview of the data collection over time within the five NBS phases. Participants of NBS-1 were invited to participate in subsequent NBS phases if they had given permission to be approached for further research and were eligible for inclusion.

FFQ=food frequency questionnaire, N=number, NIMA=non-invasive measurements of atherosclerosis, QN=questionnaire, y=year.

Age above 70 years

Of the 2253 participants aged above 70 years who had given permission to be approached for further research and were still alive, 1596 filled out the NBS-2 QN (71%).

NBS-3

In 2008-10, NBS-3 was carried out to obtain more detailed information about the nutritional status. A total of 5363 people were invited to fill out a food frequency questionnaire (FFQ). This FFQ was a validated instrument developed by the Division of Human Nutrition at Wageningen University.[1,2] Crude questionnaire data were converted to nutrient intake using the Dutch Food Composition Table from 2006 (NEVO 2006); these data are available for 2506 participants (47%).

NBS-4

In 2008 NBS-4 was started, to increase compatibility and similarity in available (risk factor) data between the NBS and cancer patient groups that were frequently studied by our research group, as well as to collect trait data and health information for a broader range of studies. A new questionnaire (NBS-4 QN) was sent out to 8109 persons who had given permission to be approached for further research and were still alive, of whom 5613 (69%) responded.

NBS-5

The NBS-5-phase was conducted in 2012 in order to collect reference data for a study on risk factors for melanoma and to obtain data on pain, dyslexia and more extensive information on physical activity. Of the 7567 persons who had given permission to be approached for further research and were still alive, 3833 (51%) returned their questionnaire (NBS-5 QN).

What has been measured?

Questionnaires

The NBS-1 QN contained questions on, among other topics, demographics, health status, lifestyle and medical

history. The NBS-2 QN covered topics about health and disease, pregnancy, mood and behaviour, daily activities and memory. The NBS-2-NIMA1, 2, and 3 QNs contained questions on general health, medical history (with a focus on cardiovascular traits), use of medication, lifestyle, family history of cardiovascular traits, and quality of life. NBS-3 QN was a food frequency questionnaire. NBS-4 QN was about lifestyle factors and health and disease. In addition, questions about reading problems, mood and behaviour, and life events were included. Finally, NBS-5 QN focused on health and disease, sun exposure, physical activity, pain and reading problems. A more detailed overview of the questionnaire data collected in the different NBS phases is provided in Table 2.

Biomaterials and blood parameters

In NBS-1, 6468 participants donated a non-fasting 8.5-ml blood sample in a serum separator tube (serum sample), and one ($N \sim 5000$) or two ($N \sim 1500$) non-fasting 10-ml EDTA blood samples (plasma sample). Blood samples were taken throughout the day; time of blood sampling was recorded. Plasma and serum samples were stored at the Department of Laboratory Medicine at -40 °C until use. Cell pellets were frozen and also stored at -40 °C for future DNA isolation.

In NBS-2-NIMA1, fasting blood samples were obtained for all participants: two 8.5-ml serum tubes, two 10-ml EDTA tubes and one 10-ml heparin tube were collected. In addition, a urine sample was collected. In NBS-2-NIMA3, fasting blood samples were obtained again: one 10-ml serum separator tube, two 10-ml EDTA tubes and one 10-ml heparin tube. In addition, urine and faeces samples and swabs from mouth, hand, foot and back for microbiome isolation, as well as adipose tissue biopsies (from abdomen and thigh) were collected. Haematological and biochemical parameters that have been measured in the NBS blood samples are presented in Table 3.

Genomics

Initially, genome-wide genotyping was carried out for 1980 samples using the Illumina HumanHapCNV370-Duo BeadChip platform, of which 1819 survived quality control (QC). Currently, we have genome-wide genotype data measured with the Illumina HumanOmniExpress-12 and -24 BeadChip available for 5363 samples; 5292 of these passed a call rate threshold of 95% and were imputed using the 1000 Genomes phase1 v3[14] together with Genome of The Netherlands (GoNL) release 5[15] data as reference. Pre-imputation QC on the marker level consisted of a minor allele frequency (MAF) > 0.01, Hardy-Weinberg equilibrium (HWE) P-value $> 10^{-4}$ and a single nucleotide polymorphism (SNP) yield $> 95\%$, resulting in 609 046 SNPs to be used in the imputation process. Imputation was performed using the Impute2 pipeline developed by the GoNL team; see [http://www.bbmriwiki.nl/wiki/Impute2Pipeline].[16] This resulted in 20 011 335 SNPs. Post-imputation quality control consisted of exclusion of population outliers using principal component analysis, exclusion of sex discrepancies based on a comparison of genotype data and clinical data, and a relatedness check, which resulted finally in 4745 samples available for genome-wide analyses.

In addition, 1931 NBS samples have been chipped with the Illumina HumanExome BeadChip, which covers putative functional exonic variants and is focused on the measurement of rare (MAF $< 0.5\%$) and low-frequency ($0.5\% <$ MAF $< 5\%$) variants [http://genome.sph.umich.edu/wiki/Exome_Chip_Design]. A total of 242 901 variants were measured with this chip, and data were called both in GenomeStudio and with zCall, a caller specifically designed for calling rare variants.[17] After quality control, that is sample call rate $> 95\%$, removal of sex discrepancies, a relatedness check, a heterozygosity check, exclusion of population outliers, marker call rate $> 98\%$, exclusion of markers that should be treated with caution–see Exome Chip Design Wiki: [http://genome.sph.umich.edu/wiki/Exome_Chip_Design]–and HWE $> 10^{-6}$, a set of 1825 samples and 242 703 markers are available for association analysis.

Linkage to the Netherlands Cancer Registry

The NBS database has been linked to the database of the Netherlands Cancer Registry in order to obtain official registry data on the occurrence of cancer among the NBS participants. Linkage was executed based on identifying information such as name, date of birth and date of death. Linkage has been repeated several times in order to update information on the occurrence of cancer. The latest linkage was performed in 2014, resulting in complete information on the occurrence of cancer in the NBS participants until 2012 and partly for 2013.

Linkage with the population registers of the municipality of Nijmegen

Periodically (in the beginning monthly, nowadays every 6 months), we receive updates from the municipality of Nijmegen about changes in vital status or address of the NBS participants who still live in the original catchment area. In this way, we have an up-to-date database of the latest contact information and vital status of the NBS

Table 2. Overview of data collected in the different NBS phases. Pdf documents of the questionnaires (in Dutch) and a detailed file with all variables that are available (in both Dutch and English) can be found at [www.nijmegenbiomedischestudie.nl]

	Examples of topics included	NBS-1[e]	NBS-2	NBS-2-NIMA1	NBS-2-NIMA2	NBS-2-NIMA3	NBS-3	NBS-4	NBS-5
General information									
Demographic data	Date of birth, gender, marital status, household composition	X	X			X			X
Anthropometry	Height, weight	X	X	X	X	X			
Ethnicity	Country of birth of participant, father, mother, race					X		X	
Education	Highest level of education	X							
Work	Employment, functions (past, current), hours per week	X	X			X			
Lifestyle									
Smoking/smoking history	Current and past behaviour	X		X	X	X		X	
Alcohol consumption	Amount, frequency	X		X	X	X		X	
Physical activity	Type, frequency	X		X	X	X			X
	Short QUestionnaire to ASses Health-enhancing physical activity (SQUASH)[3]					X			X
Nutrition	Consumption of fruits, vegetables, dairy, coffee, drinks	X				X			
	Food-frequency questionnaire						X		
Occupational exposure	Chemicals, pesticides, fumes							X	
Sun exposure	Type of skin, sunburn, duration and frequency of sun exposure							X	X
Hair dye	Colour, frequency							X	
Health and disease									
Self-rated physical health	Headache, sore throat, painful joints	X	X			X		X	X
	Items of the Short Form (36) Health Survey (SF-36)[4]	X							
Disability/mobility in activities of daily living	Walking the stairs, washing, dressing	X	X[a]			X			
Blood donation	Frequency	X						X	
Fatigue	Frequency, duration, influence on daily activities	X							
Diseases diagnosed by a physician, e.g. cardiovascular diseases, lung diseases, neurological disorders	Diagnosis, age at diagnosis, under treatment	X	X	X[d]	X[d]	X[d]			X
Medication use	Medication, ever and current use	X		X	X	X		X	
Use of vitamins	Vitamins used for minimally 6 months, ever and current use	X	X						
Medical history of family		X	X	X[d]	X[d]	X		X	

(continued)

Table 2. Continued

	Examples of topics included	NBS-1[e]	NBS-2	NBS-2-NIMA1	NBS-2-NIMA2	NBS-2-NIMA3	NBS-3	NBS-4	NBS-5
	Date of birth, age at death, ever diagnosed with cancer: mother, father, children, brothers, sisters; family history of certain diseases, e.g. cardiovascular disease, fertility problems, kidney diseases								
Psychosocial parameters									
Self-rated mental health	Memory problems, mood symptoms	X	X			X			
	Items of the Short Form (36) Health Survey (SF-36)[4]	X							
Psychological problems and symptoms	Diagnosis of diseases, family history		X					X	
	Beck Depression Inventory (BDI)-II[5]		X						
	Combination of Autism Spectrum Quotient Test (AQ test)[6] items and DSM-IV items for autism spectrum disorders[c]		X						
	DSM-IV ADHD Rating Scale[7]		X						
	Symptom Checklist (SCL-90), scales: agoraphobia and anxiety[8]		X						
	Eysenck Personality Questionnaire Revised Short Scale (EPQ-RSS)[9]		X						
	Center for Epidemiologic Studies Depression Scale (CES-D)[10]		X[a]						
Memory	Informant Questionnaire on Cognitive Decline in the Elderly (IQCODE)[11,12]		X[d]						
Reading problems	Diagnosis, symptoms as a child							X	
	Interactive Dyslexia test Amsterdam-Antwerpen - MBO (IDAA-MBO)[b]								X
Life events	List of Threatening Life Events,[13] age							X	
Pain	Duration (temporary/chronic), where								X
Reproduction									
Pregnancy and fertility (men and women)	Number of pregnancies, time till pregnant, outcome of pregnancies, fertility treatment, lifestyle and environmental exposure in relation to getting pregnant	X	X[f]						
Questions for females									
Menstruation	Age at menarche, menstruation pattern, use of birth control pill, uterus and ovaries	X						X	
Menopause	Presence of menopause, use of hormone replacement therapy	X						X	

(continued)

Table 2. Continued

	Examples of topics included	NBS-1[e]	NBS-2	NBS-2-NIMA1	NBS-2-NIMA2	NBS-2-NIMA3	NBS-3	NBS-4	NBS-5
Pregnancy and fertility	Children, breastfeeding, fertility treatment, hormones							X	
Health and disease	Mammography, X-rays, diseases of female reproductive system							X	
Questions for males									
Hair pattern, acne, prostate	Body hair, head hair, acne characteristics, prostate abnormalities							X	

The first phase of NBS (NBS-1) has been followed by four additional phases (NBS-2, -3, -4 and -5) across the period 2002-2016. NBS-NIMA1, -2 and -3 are sub-studies of NBS-2 focused on cardiovascular risk prediction using non-invasive measurements of atherosclerosis (NIMA).

[a]Only for participants older than 70 years.

[b]Dutch questionnaire.

[c]Validated in a Dutch sample ($N = 50$) (paper in preparation).

[d]Focus on cardiovascular traits.

[e]The NBS database has been linked to the database of the Netherlands Cancer Registry and thus also contains official registry data on the occurrence of cancer among the NBS participants; latest linkage covers cancer registry data until 2013.

[f]Not for participants older than 70 years

participants. This linkage does not provide information on causes of death, neither can we obtain these data due to a lack of informed consent.

What has it found? Key findings and publications

The first paper based on NBS data was published in 2006, which was about thyroid function and presence of anti-thyroperoxidase antibodies in the NBS.[18] Up to 22 June 2016, there have been 171 peer-reviewed articles published that used data from the NBS. An up-to-date list can be found on our website [www.nijmegenbiomedischestudie.nl]. The papers cover a wide range of topics, as there is a large variety of demographic, clinical, biochemical and genetic variables available. The project team of the NBS itself is focused on research into bladder cancer, atherosclerosis and cardiovascular risk factors, disorders of iron metabolism and psychiatric disorders in the adult population (like attention deficit/hyperactivity disorder (ADHD) and autism spectrum disorders).

The NBS has contributed genetic data to many international consortia that studied genome-wide DNA variation in relation to binary disease phenotypes, using the NBS participants as a control group, and quantitative traits. This has led to the identification of novel associations between DNA variants and a whole range of traits and diseases, for example urinary bladder cancer,[19–22] schizophrenia[23] and educational attainment.[24] Furthermore, smaller-sized genetic studies that zoomed in on specific candidate genes have been performed using NBS data.[25–27] Also, genetic variation has been used to draw conclusions about causality of risk factor-outcome associations. An example is a Mendelian randomization study that indicated that iron traits might play a role in atherosclerosis.[28]

Phenotypic data from NBS have also been used in many epidemiological studies. Reference values have been constructed for thyroid function,[18] glomerular filtration rate[29,30] and hepcidin.[31] In addition, data from the NBS-2-NIMA sub-study have been exploited to study risk factors and risk prediction models for atherosclerosis and cardiovascular traits.[32–37] This revealed, for example, that waist circumference is independently associated with subclinical atherosclerosis.[36] Finally, NBS has been used as a control group for studies into risk factors of bladder cancer and prostate cancer.[38–40] One of these studies showed that there is no association between personal hair dye use and bladder cancer risk, also when taking various types of hair dye, intensity of exposure to hair dyes or dye colour into account.[38]

Table 3. Overview of haematological and biochemical parameters that have been measured in the NBS blood samples

Group	Parameter	NBS-1[a] ($N = 6468$)	NBS-2-NIMA1[b] blood samples ($N = 1517$)	NBS-2-NIMA2[b] blood samples ($N = 95$)	NBS-2-NIMA2 urine samples ($N = 1066$)	NBS-2-NIMA3[b] blood samples (N=302)
Lipids	Total cholesterol	X	X	X		X
	HDL-cholesterol	X	X	X		X
	LDL-cholesterol	X	X	X		X
	Triglycerides	X	X	X		X
	Apolipoprotein A1		X			
	Apolipoprotein B		X	X		X
	Adiponectin		X			
Iron status	Ferritin	X				
	Iron	X				
	Total iron-binding capacity	X				
	Transferrin saturation[c]	X				
	Hepcidin	X[d]				
	Haemoglobin					X
	Haematocrit, mean corpuscular ahemoglobin concentration (MCHC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH)					X
Metabolites	Homocysteine, cysteine, methionine, serine, glycine, cystathionine, tryptophan, kynurenine, folate, cobalamin, pyridoxal phosphate, pyridoxal, pyridoxic acid, pyridoxamine, pyridoxine, riboflavin, neopterine, cotinine, paba-glutamine, methylmalonic aciduria type A protein		X			
Thyroid function	Thyroid-stimulating hormone	X	X			
	Free T4	X	X			
	Anti-TPO	X	X			
Liver	Alanine aminotransferase	X				
	Bilirubin		X			
Inflammation	C-reactive protein	X	X			X
	High sensitive C-reactive protein		X	X		
	Macrophage migration inhibitory factor (MIF)	X[d]				
	IL-18		X			
	Macrophage colony-stimulating factor (M-CSF)		X			
	IL-1β, IL-6, IL-8, VEGF					X
	Erythrocytes, leucocytes				X	X
	Thrombocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils					X
					X	

(continued)

Table 3. Continued

Group	Parameter	NBS-1[a] ($N = 6468$)	NBS-2-NIMA1[b] blood samples ($N = 1517$)	NBS-2-NIMA2[b] blood samples ($N = 95$)	NBS-2-NIMA2 urine samples ($N = 1066$)	NBS-2-NIMA3[b] blood samples (N=302)
	Nitrite, protein, glucose, ketones, urobilinogen, bilirubin, epithelial cells, bacteria, casts					
Renal function	Creatinine	X	X		X	X
	Albumin	X	X		X	
	Urea	X				X
Diabetes	Fasting glucose		X	X		X
	Insulin					X
Iodine status	Iodine				X	

The first phase of NBS (NBS-1) has been followed by four additional phases (NBS-2, -3, -4 and -5) across the period 2002-16. NBS-NIMA1, -2 and -3 are sub-studies of NBS-2, focused on cardiovascular risk prediction using non-invasive measurements of atherosclerosis (NIMA).

[a]Samples were non-fasting and taken during the day. Time of blood sampling was recorded.

[b]Fasting samples taken in the morning.

[c]Transferrin saturation is calculated by dividing serum iron by total iron-binding capacity.

[d]Hepcidin and macrophage migration inhibitory factor have been measured in 2998 samples.

What are the main strengths and weaknesses?

The NBS was designed to obtain a universal reference population and it has been extremely valuable as such. A main strength of NBS is that the participants are very well phenotyped, with data on a wide variety of variables, phenotypical, environmental and biochemical; NBS thus provides a very rich source of information on a broad range of research questions. In addition, data collection in consecutive phases has allowed collection of information on additional topics that were not covered in NBS-1. Besides, the NBS database has been linked to databases of the Netherlands Cancer Registry, increasing the amount of data available for analysis even further. The NBS is also a relatively large study population, containing questionnaire data on almost 10 000 participants and genomics data for more than 5000 participants. Finally, contact information and vital status of the NBS participants living in the original catchment area have been kept up to date using information on mutations from the register of the municipality of Nijmegen which is sent to us on a regular basis. NBS participants are thus approachable for future research as a source population for new studies. For example, NBS is currently participating in the Biobank Netherlands Internet Collaboration (BIONIC) study [http://www.emgo.nl/research/cross-campus-collaborations/research-projects/1454/bionic-biobank-netherlands-internet-collaboration-proof-of-principle-for-major-depressive-disorder/background/]: 3684 NBS participants were invited and 1510 have responded so far (41%).

All participants of the NBS were inhabitants of the municipality of Nijmegen at the time of inclusion. Thus, the NBS represents the population of the eastern part of The Netherlands. How well the NBS represents the source population and the Dutch population has not formally been studied. It is known, however, that participants in the NBS are relatively highly educated, based on informal comparisons with the education level of the total Dutch population and of case series that are being studied by the NBS project team. In addition, it is important to realize that the age distribution of the NBS is not representative of the age distribution of the population of the municipality Nijmegen, as the NBS sample was drawn from the register of the population of the Nijmegen stratified by 5-year age groups. In order to draw conclusions about prevalence of diseases, the sampling fraction for each age group should thus be taken into account. Sampling fractions are available on request.

Importantly, the NBS is a fixed cohort and not a dynamic population. The NBS cohort is consequently getting older over time, and numbers of participants in NBS

decrease due to death of the included subjects. In addition, the subset of participants who contributed to later phases of NBS, that is NBS-2 to NBS-5, represents most probably a selected group of people, which should be taken into account when thinking about the generalizability of study outcomes to the general population using data from later phases of NBS.

Finally, blood samples collected in NBS-1 were taken non-fasting and not at a fixed time point during the day. This is important for some of the biochemical measurements, such as the blood lipids and the iron parameters, but can be taken into account by using recorded time of blood sampling in statistical analysis.

Can I get hold of the data? Where can I find out more?

The data of the NBS are freely available to the international scientific community. The website [www.nijmegen biomedischestudie.nl] contains information on the collected data, including pdf documents of the questionnaires (in Dutch) and a detailed file with all variables that are available (in both Dutch and English). Researchers can apply for use of the data (questionnaire data, laboratory parameters and cancer registry linkage data) for scientific projects by submitting a research plan to [info@nijmegen-biomedischestudie.nl], which should contain information on the background of the research, the research questions to be answered, an analysis plan and the data requested. Data requests will be evaluated by the NBS project team, and after approval a Data Transfer Agreement (DTA) will be made containing the conditions under which the data will be transferred. The data will be provided free of any costs, but may only be used to answer the research question(s) specified in the research plan.

In October 2013, the NBS biomaterial collection was transferred to the Radboud Biobank, an infrastructure within the Radboud university medical center for the collection, storage and management of biomaterial, and the matching to clinical data.[41] The biosamples are also available to the scientific community on a fee-for-service basis. Requests can be made to the Radboud Biobank directly, see [www.radboudbiobank.nl] for the application procedure.

Profile in a nutshell

- The NBS is a large, well-phenotyped observational population-based study that was established to obtain a universal reference population that can be used in a variety of (case-control) studies in order to study genetic variation, lifestyle and environmental exposures in relation to a variety of traits or diseases.
- The first phase of NBS was started in 2002: 22 451 inhabitants of the municipality of Nijmegen were invited, of whom 9350 filled out the questionnaire and 6468 donated a blood sample. The age ranged from 18 to 99 years.
- The initial phase of NBS has been followed by four additional phases from 2005 to 2016, including additional questionnaires and clinical and biochemical measurements.
- Contact information and vital status of the NBS participants living in the original catchment area have been kept up to date until now, using information from the demography register of the municipality of Nijmegen; more than 6000 of the participants are still approachable for future studies.
- Available data comprise a wide range of phenotypic, environmental and biochemical variables, genome-wide genetic information and linkage to the Netherlands Cancer Registry. Also, biospecimens (serum, plasma and DNA samples) can be requested.
- Data are freely available to the international scientific community; send a request to [info@nijmegenbiomedischestudie.nl]; see [www.nijmegenbiomedischestudie.nl for more information].

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