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A guide to biomarker data in the CLOSER studies

A catalogue across the cohort and longitudinal studies

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July 2017

E·S·R·C ECONOMIC & SOCIAL RESEARCH COUNCIL

MRC Medical Research Council

To cite this report, please use the following reference:
Ruiz M, Benzeval M, Kumari M. A guide to biomarker data in the CLOSER studies: A catalogue across the cohort and longitudinal studies. London, UK: CLOSER; 2017.

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Acknowledgements

This project is funded as an Innovation enhancement to a collaborative research programme entitled 'Cohorts and Longitudinal Studies Enhancement Resources' (CLOSER), by the ESRC grant reference: ES/K000357/1.

The Avon Longitudinal Study of Parents and Children, **ALSPAC**, is funded by the Medical Research Council, the Wellcome Trust and the University of Bristol. *Understanding Society*: The UK Household Longitudinal Study, **UKHLS**, is an initiative funded by the Economic and Social Research Council (ESRC) and various Government Departments, with scientific leadership by the Institute for Social and Economic Research, University of Essex, and survey delivery by the National Centre (NatCen) for Social Research and Kantar Public. The research data are distributed by the UK Data Service. The Southampton Women's Survey, **SWS**, is funded by the Medical Research Council (MRC), the University of Southampton and other sources including the Dunhill Medical Trust, the British Heart Foundation, Arthritis Research UK and the Food Standards Agency. The National Child Development Study, **NCDS**, is funded by the ESRC and is managed by the Centre for Longitudinal Studies (CLS). The MRC National Survey of Health development, **NSHD**, is funded by the Medical Research Council and managed by the MRC Unit for Lifelong Health and Ageing at UCL. The Hertfordshire Cohort Study, **HCS**, is funded primarily by the Medical Research Council, with some support from medical charities such as the British Heart Foundation and the Arthritis Research Campaign.

The following individuals provided assistance in accessing, understanding and managing data:

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UKHLS: *Understanding Society* biomarker's team

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1 Introduction

About CLOSER

Cohort and Longitudinal Studies Enhancement Resources (CLOSER[1]) is a project funded by the Economic and Social Research Council (ESRC) and the Medical Research Council (MRC) that aims to maximise the use, value and impact of longitudinal studies in the UK. CLOSER brings together eight leading studies, the British Library and the UK Data Service, to stimulate interdisciplinary longitudinal research, develop shared resources, provide training and share expertise.

The CLOSER partnership includes the following eight cohorts and longitudinal studies:

- Avon Longitudinal Study of Parents and Children (ALSPAC): *Children of the 90s*
- The UK Household Longitudinal Study (UKHLS): *Understanding Society*
- Southampton Women's Survey (SWS)
- 1958 National Child Development Study (NCDS)
- MRC National Survey of Health and Development (NSHD)
- Hertfordshire Cohort Study (HCS)
- 1970 British Cohort Study (BCS)
- Millennium Cohort Study (MCS): *Child of the New Century*

About Biomarkers

The National Institute of Health (NIH) Biomarkers Definitions Working Group[2], defined a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention". In social science, biomarkers may allow researchers to investigate factors that contribute to and interact with health, social conditions and environment. When combined with longitudinal data, biomarkers can shed light on the complex interplay between biology, behaviour and the social environment over the life course for both health and other outcomes.

A number of the component studies within CLOSER have collected biological samples including blood, saliva and urine samples from which a wide array of biomarkers has been measured. The diverse age span between these participant studies makes CLOSER a rich resource for social-biological research over time, at different points in history and across the lifespan.

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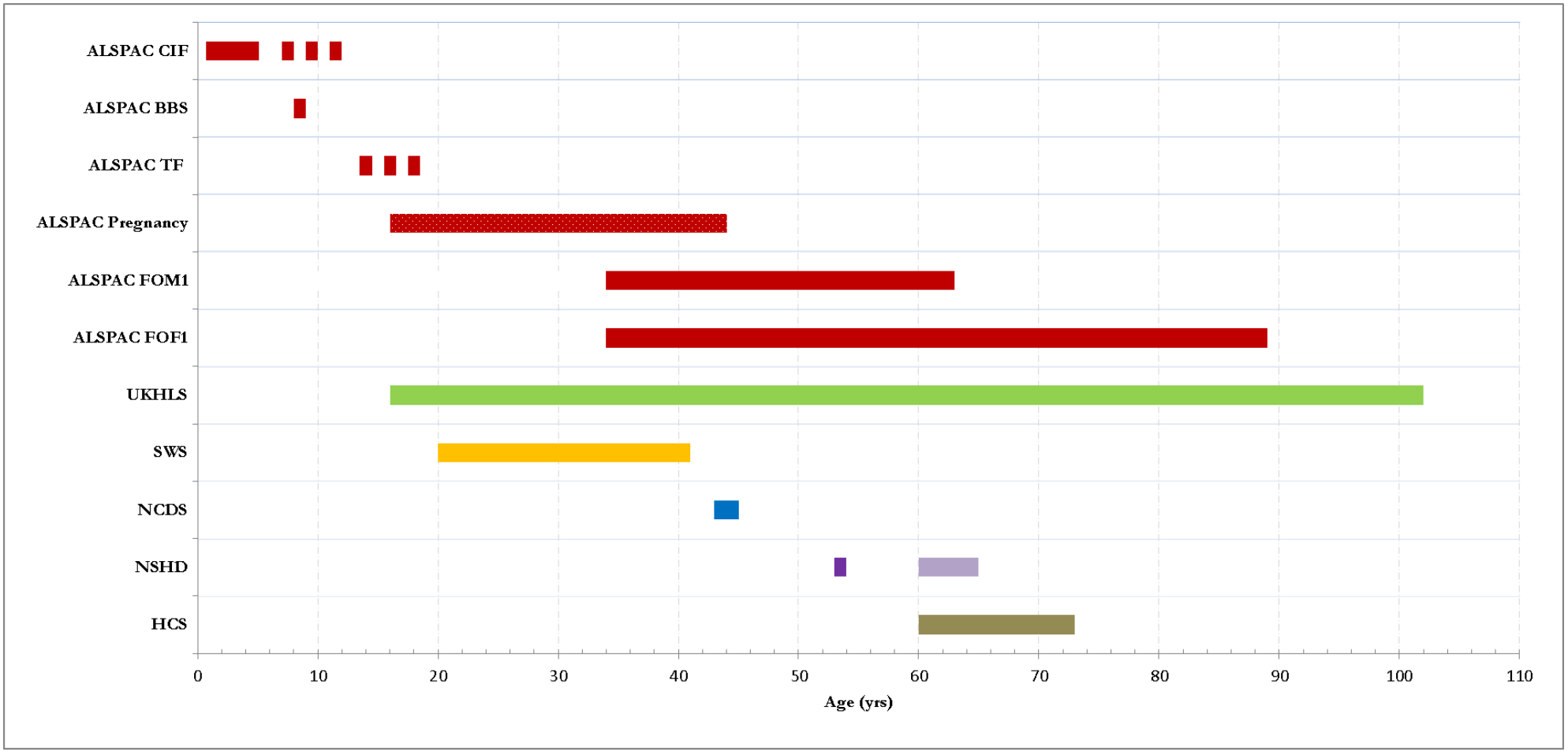
[1] http://www.closer.ac.uk/

[2] Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: Preferred definitions and conceptual framework. Clin Pharmacol Ther. 200; 69(3):89-95.doi:10.1067/mcp.2001.113989.

Here, we present a catalogue of biomarkers across CLOSER, derived from blood, saliva or urine samples, which are publicly available and accessible when data-access protocols have been completed. We pay attention to biomarkers that are relevant for research in the social-biological interface, and of interest to ESRC's strategic investment in biosocial research.[3] We first describe each study, the collection conditions, procedures used and data access protocols. We then describe each biomarker where measured by more than one study and identify issues users need to consider in analysis (see **Biomarker Glossary** section). See **Figure 1** for the age span of those CLOSER studies that have collected blood, saliva or urine samples. **Table 1** presents baseline characteristics of the CLOSER studies and **Table 2** lists biomarkers from biological samples where they have been measured in more than one of the CLOSER studies.

[3] Hobcraft, J. (2009). Reflections on the incorporation of biomeasures into longitudinal social surveys: an international perspective. *Biodemography and Social Biology,* 55(2): 252-269.

Figure 1 The age of participants and range for each of the CLOSER studies for which biomarker data have been extracted.



ALSPAC: Avon Longitudinal study of Parents and Children, **CIF:** Children in Focus, clinics for babies and toddlers from 8 months up to 61 months and Focus clinics for older children at 7 yrs, 9 yrs, 11+ yrs and the **BBS (**Before Breakfast Study at 8 yrs); **TF:** Teen Focus, focus clinics for teenagers at 13.5 yrs (**TF2**), 15.5 yrs (**TF3**) and 17.5 yrs (**TF4**); **FOM1:** Focus on Mothers, first follow up focus clinic for mothers; **FOF1:** Focus on Fathers, first follow up focus clinic for mother's partners; **UKHLS**: UK Household Longitudinal study, **SWS**: Southampton Women's Survey; **NCDS**: National Child Development Study; **NSHD**: National Survey of Health and Development; **HCS**: Hertfordshire Cohort study.

Table 1 Characteristics of CLOSER studies that have collected blood, urine and /or saliva samples.

COHORT	Participants with Biological data, N		Clinic dates	Mean age (age range)	Setting	
					Home	Clinic
ALSPAC*	**CIF** at 8 mths	1 314	Feb/1993 - Aug/1993	8.8 mths (32-42 weeks)	-	*Non-fasting* blood
	CIF at 12 mths	1 241	Jun/1993 - Dec/1993	13.4 mths (51-62 weeks)	-	*Non-fasting* blood
	CIF at 18 mths	1 183	Dec/1993 - Jun/1994	20.0 mths (76-87 weeks)	-	*Non-fasting* blood
	CIF at 31 mths	1 135	Jan/1995 - Jul/1995	33.7 mths (132-140 weeks)	-	*Non-fasting* blood
	CIF at 43 mths	1 065	Jan/1996 - Jul/1996	46.9 mths (184-196 weeks)	-	*Non-fasting* blood
	CIF at 61 mths	994	Jul/1997 - Apr/1998	67.2 mths (260-292 weeks)	-	*Non-fasting* blood
	BBS at 8 yrs	830		8.2 yrs (8.0-8.5 yrs)	-	*Fasting* blood[1]
	F7 at 7 yrs	8 297	Sep/1998 - Sep/2000	7.5 yrs (90.5-82 mths)	-	*Non-fasting* blood
	F9 at 9 yrs	7 725	Jan/2001 - Jan/2003	9.9 yrs (118.5-105 mths)	-	*Non-fasting* blood
	F11+ at 11 yrs	7 159	Jan/2003-Jan/2005	11.7 yrs(125-163 mths)	-	*Non-fasting* blood
	TF2 at 13.5 yrs	6 147	Jan/2005 - Sep/2006	13.8 yrs (150-182 mths)	-	*Non-fasting* blood
	TF3 at 15.5 yrs	5 509	Oct/2006 - Nov/2008	15.5 yrs (171-212 mths)	-	*Fasting* blood[1]; saliva; urine
	TF4 at 17.5 yrs	5 081	Dec/2008 - Jun/2011	17.9 yrs (195-241 mths)	-	*Fasting* blood[1], urine
	FOM1	4 832	Dec/2008 - Jul/2011	48 yrs (34-63 yrs)	-	*Fasting* blood[1]
	FOF1	2 001	Sep/2011 - Feb/2013	53 yrs (34-89 yrs)	-	*Fasting* blood[1]
	Pregnancy†	7 501	1990-1993	28 yrs (16-44 yrs)	-	*Non-fasting* blood
UKHLS	13 107		2010-2012	52 (16-102) yrs	*Non-fasting* blood	-
SWS**	**Init**	8 755	1998-2002	28 (19-36) yrs	*Non-fasting* blood; urine; saliva	-
	EP	2 317	1998-2007	30 (20-41) yrs	*Non-fasting* blood	
	LP	2 032	1998-2007	30 (21-41) yrs	*Non-fasting* blood	
	Father of baby	1 762	1998-2002	-	*Non-fasting* blood; saliva	
NCDS	8 018		2001-2003	44 (44-46) yrs	*Non-fasting* blood; saliva	-
NSHD	at 53 yrs	3 035	1999	53 (53-54) yrs	*Non-fasting* blood	-
	at 63 yrs	2 229	2006-2010	63 (60-65) yrs	*Fasting* blood[2]; urine; saliva	*Fasting* blood[2]; urine; saliva
HCS	2 997		1998-2004	66 (60-73) yrs	-	*Fasting* blood[3]; urine; saliva

***CIF:** Children in Focus, clinics f or babies and toddlers; **BBS:** Before Breakfast Study; **F7, F9, F11+:** Focus clinics for older children at specified age; **TF:** Teen Focus, focus clinics for teenagers at specified age; **FOM1:** Focus on Mothers, first follow up focus clinic for mothers; **FOF1:** Focus on Fathers, first follow up focus clinic for mother's partners.

† Pregnancy blood samples in ALSPAC were taken at varying gestation stages (look at individual biomarkers for further information).

****Init:** Initial interview carried out before pregnancy; **EP**: Early pregnancy, measured at approximately 11 weeks' gestation; **LP**: Late pregnancy, measured at approximately 34 weeks gestation.

[1] Participants were asked to fast for a minimum of 6 hours overnight (for those attending in the morning) or for a minimum of 6 hours before attending clinic after lunch time

[2] Participants were asked to fast from 22:00 hours on the night before the visit, and the majority of blood samples were collected between 08:00 and 09:00 hours the following day

[3] Participants were asked to fast for 12 hours, overnight

Table 2 List of biological markers available in more than one study in CLOSER. All biomarkers in this list have been measured from blood samples, unless otherwise noted.

System	Biomarker	ALSPAC	UKHLS	SWS	NCDS	NSHD	HCS
Metabolic	Cholesterol, Total	✓	✓	✓	✓	✓	✓
	Cholesterol, High Density Lipoprotein, HDL	✓	✓		✓	✓	✓
	Cholesterol, Low Density Lipoprotein, LDL	✓	✓		✓	✓	✓
	Apolipoprotein A1, Apo A1	✓					✓
	Apolipoprotein B, Apo B	✓					✓
	Lipoprotein[a], Lp[a]					✓	✓
	Triglycerides	✓	✓		✓	✓	✓
	Glucose	✓				✓	✓
	Insulin	✓				✓	✓
	Proinsulin	✓				✓	✓
	Glycated Haemoglobin, HbA1c	✓	✓		✓	✓	
Diet & Iron Markers	Ferritin	✓	✓	✓		✓	
	Haemoglobin, Hb	✓	✓	✓		✓	
	Vitamin C			✓		✓	
	Vitamin D	✓		✓		✓	
Inflammatory	C-Reactive Protein, CRP	✓	✓		✓	✓	✓
	Fibrinogen		✓		✓		
	Immunoglobulin E, IgE	✓			✓		
	Interleukin-6, IL-6	✓				✓	
	Red Cell Folate, RCF			✓		✓	
	Tissue Plasminogen Activator, TPA				✓	✓	
	Von Willebrand factor, VWF				✓	✓	
	Platelet count			✓		✓	
	Red Blood Cell, RBC, count			✓		✓	
	White Blood Cell, WBC, count			✓		✓	
Neuro-Endocrine	Cortisol (blood)	✓					✓
	Cortisol (saliva)	✓			✓	✓	✓
	Dehydroepiandrosterone Sulphate, DHEAS	✓	✓			✓	
	Insulin-like Growth Factor 1, IGF-1	✓	✓		✓	✓	
	Insulin-like Growth Factor 1, IGF-2	✓				✓	
	Insulin-like Growth Factor Binding Protein 3, IGFBP3	✓				✓	
	Sex Hormone Binding Globulin, SHBG	✓				✓	
	Testosterone	✓	✓			✓	
Kidney & Liver	Calcium	✓		✓		✓	
	Creatinine (blood)		✓			✓	
	Creatinine (urine)			✓		✓	✓
	Phosphate			✓		✓	
	Urea		✓			✓	
	Alanine Transaminase, ALT	✓	✓			✓	
	Alkaline Phosphatase, ALP		✓	✓		✓	
	Aspartate Transaminase, AST	✓	✓			✓	
	Albumin	✓	✓	✓		✓	
	Gamma Glutamyl Transferase, GGT	✓	✓			✓	

2 BIOMARKERS in the studies included in CLOSER

2.1 ALSPAC: Avon Longitudinal Study of Parents and Children: *Children of the 90s*

"*Children of the 90s*", the Avon Longitudinal Study of Parents and Children (ALSPAC) is a birth cohort study investigating influences on health and development across the life course. It considers genomic, biological, psychological, social and other environmental exposures in relation to a diverse range of developmental and health outcomes. Information can be found in two cohort profile papers[4,5].

All pregnant women resident in a defined area in the South West of England, with an expected date of delivery between 1st April 1991 and 31st December 1992, were eligible to join the study and 14 541 pregnant women were recruited. Biological samples were collected including maternal blood and urine, umbilical cord blood, placentas, paternal blood and saliva and children's blood, saliva and urine. Women have been followed up since recruitment, and have completed numerous questionnaires. Follow-up clinics (Focus on Mothers, FOM1-FOM4) of all mothers still engaged with the study have been completed that have included the collection of blood samples from fasted participants. Measurements were selected to record the antecedent exposures of future health and psychosocial outcomes. The index children of recruited pregnancies completed follow-up questionnaires and clinical assessment visits (Children in Focus, CIF; Focus clinics, F7, F9; Teen Focus, TF). In addition to biological samples, genetic (DNA on 11 343 children, genome-wide data on 8365 children, complete genome sequencing on 2000 children) and epigenetic (methylation sampling on 1000 children) information and linkage to health and administrative records is available.

ALSPAC Biological samples collection

Biological samples from pregnant women, partner and child were collected at various points in time (**Table 1**). Participants were invited to special clinics where all biological samples were collected. During pregnancy, participants were not requested to fast and bloods and urine samples were collected. During follow-up clinics, participants were asked to fast and

[4]Boyd et al. International Journal of Epidemiology 2012;1–17
[5]Fraser et al. International Journal of Epidemiology 2013;42:97–110

blood samples from the mother (Focus on Mothers clinics, FOM1) and her partner (Focus on Fathers clinics, FOF1) was collected. Children provided biological samples during special focus clinics at various ages, from birth (Children in Focus clinics, CIF); Focus clinics at 7, 9 and 11 yrs (F7, F9, and F11^{+}) and clinics from teen-age up to 18 years (Teens in Focus clinics, TIF).

The ALSPAC cohort profile with an extensive list of biomarkers for child, mother and mother's partner has been published elsewhere[2, 3]; the following **Table 3** is a concise list of the biomarkers available from ALSPAC.

ALSPAC Data Access

Access to ALSPAC research data must be requested using the procedures described in the ALSPAC access policy[6] and is subject to eligibility, the ALSPAC funder's terms and conditions and University of Bristol policies and procedures. To use existing biological samples or to carry out specific genotyping on ALSPAC DNA, an online research proposal form describing the proposed research needs to be completed (https://proposals.epi.bristol.ac.uk/).

The ALSPAC website (http://www.bristol.ac.uk/alspac/researchers/our-data/questionnaires/provides) provides copies of the questionnaires and documentation used in the study. The website also provides a data dictionary and a variable catalogue, to help users make a formal request for data. Further research metadata can be accessed via the MRC Gateway Portal (https://www.datagateway.mrc.ac.uk/) and the CLOSER Discovery platform (https://discoverycloser.ac.uk/).

All researchers accessing ALSPAC data will be charged on a cost recovery basis, as detailed in the ALSPAC access policy[4].

[6] http://www.bristol.ac.uk/media-library/sites/alspac/documents/ALSPAC_access_policy.pdf

Table 3. Concise list of biomarkers available in the ALSPAC dataset for mothers and children. Biomarkers highlighted in bold are unique to the ALSPAC dataset.

System	Maternal Biomarkers [1]	Children Biomarkers [2]
Metabolic	Cholesterol, Total (mmol/L) Cholesterol, High Density Lipoprotein, HDL (mmol/L) Cholesterol, Low Density Lipoprotein, LDL (mmol/L) **Cholesterol, Very Low Density Lipoprotein, VLDL (mmol/L)** Glucose (mmol/L) Insulin (pmol/L) Proinsulin (pmol/L) Fatty acid profile (µg/mL)***** Triglycerides (mmol/L)	Cholesterol, Total (mmol/L) Cholesterol, High Density Lipoprotein, HDL (mmol/L) Cholesterol, Low Density Lipoprotein, LDL (mmol/L) **Cholesterol, Very Low Density Lipoprotein, VLDL (mmol/L)** Adiponectin (µg/mL)****** **Apolipoprotein A, Apo A (g/L)** Apolipoprotein A1, Apo A1 (g/L) Apolipoprotein B, Apo B (g/L) Fatty acid profile (µg/mL)***** **Free Fatty acids (mmol/L)** Leptin (ng/mL)****** Triglycerides (mmol/L) Glucose (mmol/L) **Post-glucose glucose (mmol/L)** **Post-glucose insulin (mU/L)** Insulin (pmol/L) Proinsulin (pmol/L) Glycated Haemoglobin, HbA1c (mmol/mol) **C-peptide**
Diet and Iron Markers	Haemoglobin, Hb (g/L) Vitamin D, D_2, D_3 (mg/mL)	Ferritin (mg/mL) Haemoglobin, Hb (g/L) **Haptoglobin (g/L)** Vitamin B12‡ Vitamin D, D_2, D_3 (mg/mL)
Inflammatory	C-Reactive Protein, CRP (mg/L) **Helicobacter pylori status**	C-Reactive Protein (mg/L) Immunoglobulin, IgE (KU/L) **Immunoglobulin G, IgG** Interleukin 6, IL-6 (pg/mL) **Auto-antibodies** C-Terminal cross linking telopeptide, CTX (ng/mL)******* **Epstein barr status (EBV)** **Chlamydia** **Gonorrhoea** **Helicobacter pylori status**
Neuro-Endocrine	Anti-Mullerian hormone, AMH***** Sex Hormone Binding Globulin, SHBG (nmol/L) Insulin Growth Factor 1, IGF-1 (ng/mL) Insulin Growth Factor 2, IGF-2 (ng/mL) Insulin Growth Factor Binding Protein 3, IGFBP3 (ng/mL) Testosterone (nmol/L) Thyroid Function Tests****** **Estradiol** **Follitropin** **Lutropin** **Phytoestrogens**	Anti-Mullerian hormone, AMH (ng/mL)***** Cortisol (mmol/L) Dehydroepiandrosterone Sulphate, DHEAs (µg/dL) **Growth hormone binding protein (ng/mL)** Insulin Growth Factor 1, IGF-1 (ng/mL) Insulin Growth Factor 2, IGF-2 (ng/mL) **Insulin Growth Factor 2 Receptor, IGF2r (ng/mL)** **Insulin Growth Factor Binding Protein 1, IGFBP1 (ng/mL)** Insulin Growth Factor Binding Protein 3, IGFBP3 (ng/mL) Sex Hormone Binding Globulin, SHBG (nmol/L) Testosterone (nmol/L) Thyroid Function Tests****** **Parathyroid hormone** **Androstenedione (ng/dL)**
Kidney & Liver	Calcium (mmol/L) Creatinine**†** Phosphate (mmol/L)**†** Protein (g/L)****** Albumin (g/L) Globulin (g/L)****** Alkaline Phosphatase, ALP (U/L)**†**	Calcium (mmol/L) Creatinine**†** Phosphate (mmol/L)**†** Potassium (blood)****** Protein (blood) (g/L)****** Sodium (urine)****** Urea**†** Alanine Transaminase, ALT (U/L) Alkaline Phosphatase, ALP (U/L)**†** Aspartate Transaminase, AST (U/L) Albumin (g/L) Globulin****** Gamma Glutamyl Transferase, GGT (U/L) Bilirubin (g/L)****** **Hepatitis A status**
Others	**Aromatic Amines** **Atrazines** **Bisphenol-A** **Cotinine (ng/mL)** **Genistein** **Daidzein** **Enterol** **Equol** **Metals (iodine, trace metals)** **Dichloroanilines** **Tryclosan** **Paracetamol**	**Metals (Deuterium, Lead, Mercury, trace metals)** **Cotinine (ng/mL)**

[1]Maternal biomarkers measured at Focus clinics. [2]Children biomarkers measured at varying ages. *Also measured in SWS, but not included in this catalogue. **Also measured in NSHD, but not included in this catalogue. ***Also measured in HCS, but not included in this catalogue. †measured but not available at present, also measured in other CLOSER studies, as described in this document. ‡for small sub-sample (~300 participants).

2.2 UKHLS: The UK Household Longitudinal Study: *Understanding Society*

The UK Household Longitudinal Study is a large longitudinal survey of households in the United Kingdom, it studies 21st century UK life and how it is changing. Every year, it captures important information about the social and economic circumstances, attitudes, behaviours and health of people living in up to 40 000 UK households.

Information is collected on everyone in a household; all young people aged 10-15 are asked to complete a self-complete questionnaire; and, all adults 16 and over are invited to take part in an interview, parents are asked question on pregnancy and birth and children under 10. Members of households recruited at the first round of data collection are interviewed each year, whether or not they have stayed part of the household, to collect information on changes to their household and individual circumstances.

In 2010-2012 (waves 2 and 3), after the annual survey, adult respondents were also invited to take part in a nurse health assessment interview, which included a range of physical measures and blood samples. With consent, the blood samples were frozen for future analysis and DNA extracted. Blood samples have been analysed to produce a set of biomarkers that are either a measure of key risk factors for diseases and/or reflect key biological pathways between social and environmental factors and health.

Of the 35 937 respondents eligible for the nurse health assessment, 20 700 took part. Of those participating in the nurse health assessment, 1 579 (7.6%) were ineligible to give blood, and a further 22.6% (4 688 people) refused. Of those eligible and consenting to give blood samples to be stored for future analysis, samples were obtained and successfully processed (at least one biomarker available) for 13 107 respondents.

UKHLS Biological samples collection

Blood samples were taken during a nurse assessment at a home visit (**Table 1**). Participants were not asked to fast before the nurse health assessment interviews, which were conducted by NatCen.

A summary of the biomarkers currently available in *Understanding Society* and some of the factors that require consideration in their analysis is published in the *Biomarker User guide and Glossary in Understanding Society*[7]. Full details about the nurse visit, and the other data collected as part of this, can be found in the Nurse Health Assessment User Guide[8], CAPI programme[9] and fieldwork protocols[10]. The following **Table 4** is a summary of biomarkers available at present in *Understanding Society*.

Table 4 Biomarkers available in the UKHLS dataset. Biomarkers highlighted in bold are unique to the UKHLS dataset.

System	Biomarker
Metabolic	Cholesterol, Total (mmol/L)
	Cholesterol, High Density Lipoprotein, HDL (mmol/L)
	Cholesterol, Low Density Lipoprotein, LDL (mmol/L)
	Triglycerides (mmol/L)
	Glycated Haemoglobin , HbA1c (mmol/mol)
Diet & Iron Markers	Ferritin (ng/mL)
	Haemoglobin, Hb (g/L)
Inflammatory	C-Reactive Protein, CRP (mg/L)
	Cytomegalovirus seropositivity, CMV (IgG-IgM)
	Fibrinogen (g/L)
Neuro-Endocrine	Dehydroepiandrosterone sulphate , DHEAS (µmol/L)
	Insulin-like Growth Factor 1, IGF-1 (nmol/L)
	Testosterone (nmol/L)
Kidney & Liver	Creatinine (blood) (µmol/L)
	Urea (mmol/L)
	Alanine Transaminase, ALT (IU/L)
	Alkaline Phosphatase, ALP (IU/L)
	Aspartate Transaminase, AST (IU/L)
	Albumin (g/L)
	Gamma Glutamyl Transferase, GGT (IU/L)

[7] https://www.understandingsociety.ac.uk/d/154/7251-UnderstandingSociety-Biomarker-UserGuide-2014.pdf?1418057881
[8] McFall S.L. et al. Understanding Society –UK Household Longitudinal Study: Waves 2 and 3 Nurse Health Assessment, 2010-2012, Guide to Nurse Health Assessment. 2014. https://www.understandingsociety.ac.uk/documentation/health-assessment
[9] https://www.understandingsociety.ac.uk/documentation/health-assessment/questionnaires
[10] NatCen (2010) Nurse Protocols for Measurements and samples used by the National Centre for Social Research, London: NatCen. Understanding Society and NatCen (2010) Understanding Society Nurse Visit Nurse Project Instructions. https://www.understandingsociety.ac.uk/documentation/health-assessment/fieldwork-documents

UKHLS Data Access

Understanding Society, UK Household Longitudinal Study can be accessed by *bona fide* researchers through the UK Data Service at the University of Essex. Data from Waves 2 and 3 of UKHLS, which includes available biomarker data, are archived at the UK Data Archive (UKDA) and can be accessed by *bone fide* researchers from across the world under the End User Licence; anyone wishing to access the data will need to register with the UK Data Service before downloading. Potentially more disclosive information (e.g. full medication details, geographic identifiers) is available under Special Licence or via the UK Data Service Secure Lab. Access for genetic data that are not linked to phenotype information is available through the European Genome-phenome Archive[11] (EGA); linked data are available through the METADAC (www.metadac.ac.uk).

The *Understanding Society* website provides research metadata, including basic frequencies, information about the questionnaires and documentation used in the study and research findings. Further metadata, survey questions and variables can be searched at the online CLOSER Discovery platform (https://discoverycloser.ac.uk/).

[11] www.ebi.ac.uk/ega

2.3 SWS: Southampton Women's Survey

The Southampton Women's survey (SWS) started in 1998 to explore how mother's dietary and lifestyle factors, before and during pregnancy, influence the health of their offspring. It was established in order to measure the pre-pregnant characteristics of women aged 20-34 years living in the city. Between April 1998 and October 2002, it recruited 12 500 women to the SWS, and followed through their subsequent pregnancies. Their 3 000 live-born infants are being followed through childhood. The SWS is the only population-based study in the developed world of a large, representative group of women who were characterised before pregnancy and had longitudinal measurements of foetal growth rates during pregnancy. Approximately two thirds of participants that were interviewed gave blood samples. About 3 000 of the women went on to become pregnant within the study and measurements from the roughly two thirds which provided blood samples were taken. Some 2 300 women gave blood sample in late pregnancy.

SWS Biological sample collection

Of those women contacted about the study, 12 579 (75%) agreed and were interviewed at home by a research nurse. All interviews were conducted between April 1998 and October 2002. If a woman was willing to take part, an appointment was made for a SWS nurse to visit her at home where blood was collected at two stages of pregnancy (Early Pregnancy at approximately 11 weeks and during Late Pregnancy at around 34 weeks) (**Table 1**). Women were not asked to fast before blood sampling. Blood, saliva and urine samples were also collected at recruitment, before pregnancy (Initial Interview). Umbilical cord blood samples were collected at birth. A detailed description of the SWS has been published elsewhere[12].

The following **Table 5** summarises the biomarkers available at present in SWS.

[12] Inskip H.M. et al. Cohort profile: The Southampton Women's Survey. International Journal of Epidemiology, 2005; 35(1); 42-48

Table 5 Biomarkers available in the SWS dataset. Biomarkers highlighted in bold are unique to the SWS dataset.

System	Biomarker
Metabolic	Cholesterol, Total (mmol/L) Fatty acid profile (μg/mL)*
Diet & Iron Markers	**Beta-carotene (μmol/L)** Ferritin (ng/mL) Haemoglobin, Hb (g/L) **Iodine (urine) (μg/L)** **Serum Folate (ng/ml)** **Vitamin A (Retinol) (μmol/L)** **Vitamin B PLP (nmol/L)** **Vitamin B pyridoxic acid (nmol/L)** Vitamin C (Ascorbic acid) (μmol/L) **Vitamin B12 (pg/mL)†** Vitamin D (nmol/L) **Vitamin D binding protein (μg/mL)**
Inflammatory	Red Cell Folate, RCF (nmol/L) **Alpha-carotene (μmol/L)** **Vitamin E (Alpha-tocopherol) (μmol/L)** **Alpha tocopherol/Total cholesterol (μmol/mmol)** Basophil ($x10^9$/L) **Beta-cryptoxanthin** (μmol/L) **Choline** (μmol/L) Eosinophil ($x10^9$/L) **Lutein** (μmol/L) **Lycopene** (μmol/L) Lymphocyte ($x10^9$/L) Mean Corpuscular Volume, MCV Mean Corpuscular Haemoglobin, MCH **Mean Corpuscular Haemoglobin Concentration, MCHC** Monocyte ($x10^9$/L) Neutrophil ($x10^9$/L) **Packed Cell Volume, PCV** Platelet count ($x10^9$/L) Red Blood Cell, RBC count ($x10^9$/L) **Red Blood Cell Distribution Width, RBCDW** **Total anti-oxidant status** (mmol/L) White Blood Cell, WBC count ($x10^9$/L)
Neuro-Endocrine	Anti-Mullerian Hormone, AMH (ng/ml)*
Kidney & Liver	Calcium (mmol/L) Creatinine (urine) (mg/L) **Iodine (urine) (μg/L)** Phosphate (mmol/L) Alkaline Phosphatase, ALP (IU/L) Albumin (g/L)

***Also measured in ALSPAC, but not presented in this document**

†Measured in ALSPAC for a small sub-sample (~300 individuals)

SWS Data access

The data are accessible to *bona fide* researchers by contacting Professor Cyrus Cooper, Director of the MRC Lifecourse Epidemiology Unit at the University of Southampton, who can forward a collaborators' agreement. Research metadata can be accessed via the MRC Gateway Portal (https://www.datagateway.mrc.ac.uk/) and the online CLOSER Discovery platform (https://discovery.closer.ac.uk/).

2.4 NCDS: National Child Development Study

This ongoing cohort study involves all babies born in one week in 1958 in England, Scotland and Wales. The survey was designed to examine the social and obstetric factors associated with stillbirth and death in early infancy among the children born in Great Britain. By collecting information on various aspects of life, the NCDS has become an invaluable data source on effects of socioeconomic circumstances on health, social mobility and changes in social attitudes.

Information was collected on the family background of the mother, her pregnancy and labour, about her baby at birth and during its first week of life. Nearly 17 500 babies were studied in total. Subsequent surveys of the babies were carried out when they were 7, 11, 16, 23, 33, 42, 46, 50 and 55 years old, attempting to trace all those born in that week of the original 1958 survey – in 1965, 1969, 1974, 1981, 1991, 1999/2000, 2004/5, 2008/9 and most recently in 2013. In the first three sweeps (at ages 7, 11 and 16), the target sample was augmented to include immigrants born in the same week. Additionally, the biomarker wave of data collection was carried out when participants were aged 44/45 yrs (2002-2004).

NCDS Biological sample collection

A 'bio-medical' survey took place between September 2002 and December 2003 (PI Christine Power and David Strachan), at age 44/45, where 9 377 cohort members participated in order to learn more about how development, environment and lifestyle affect people's health (**Table 1**). Using a protocol similar to that used in UKHLS (see page 8), participants were not asked to fast before a nurse visited them in their home and blood was collected from 88% of those examined. A number of additional clinical and measures of functioning and medication information were collected. Finally, nurses left behind instructions and kits containing Sarstedt salivettes for collecting and returning 2 saliva samples. A detailed cohort profile has been published elsewhere[13]. The following **Table 6** lists biomarkers measured from blood and saliva collections.

[13] Power C. and Elliott J. Cohort profile: 1958 British birth cohort (National Child Development Study). Int J Epidemiol. 2006; 35(1):34-41.

Table 6 Biomarkers available in the NCDS dataset. Biomarkers highlighted in bold are unique to this dataset.

System	Biomarker
Metabolic	Cholesterol, Total (mmol/L) Cholesterol, High density Lipoprotein, HDL (mmol/L) Cholesterol, Low density Lipoprotein, LDL (mmol/L) Triglycerides (mmol/L) **D-dimer** (ng/mL) Glycated Haemoglobin, HbA1c (mmol/mol)
Diet & Iron Markers	**Vitamin D (saliva)** (ng/mL)
Inflammatory	C-Reactive Protein, CRP (mg/L) Fibrinogen (g/L) Immunoglobulin E, IgE† (KU/L) Tissue Plasminogen Activator, TPA (ng/mL) Von Willebrand Factor, VWF (IU/dL)
Neuro-Endocrine	Cortisol (saliva) (nmol/L) Insulin-like Growth Factor, IGF-1 (nmol/L)

† IgE data also available for specific allergens: house dust mites, mixed grasses, cat fur when total IgE is >30KU/L.

NCDS Data Access

The majority of NCDS survey data can be accessed by *bona fide* researchers through the UK Data Service at the University of Essex. As for UKHLS, anyone wishing to access the data will need to register with the UK Data Service. However, data from the biomedical sweep, including most data generated from the biological samples is available via Special License or via the UK Data Service Secure Lab (https://www.ukdataservice.ac.uk/get-data/how-to-access/accesssecurelab).

Research metadata, including basic frequencies, is available using NESSTAR at the UK Data Service. The Centre for Longitudinal Studies (CLS) website provides copies of the questionnaires and documentation used in the study. The CLS data dictionary offers further metadata including variables and frequencies. Further metadata, survey questions and variables can be searched at the online CLOSER Discovery platform (https://discoverycloser.ac.uk/).

Access to the majority of genotypes generated from NCDS participants is governed by the Wellcome Trust Case Control Consortium CDAC (https://www.wtccc.org.uk/info/access_to_data_samples.html). Access to genotypes linked to other variables or applications for access to DNA and for new uses of biological samples is via the METADAC (http://www.metadac.ac.uk/).

2.5 NSHD: National Survey of Health and Development

The MRC National Survey of Health and Development, also known as the 1946 birth cohort, is the oldest British birth cohort in the world. The NSHD offers a unique opportunity to explore the long-term biological and social processes of ageing and how ageing is affected by factors acting across the whole of life. It comprises 5 362 individuals born in England, Scotland, and Wales in one week in March 1946. Data have been collected on the individuals at 24 time points (waves), as well as through smaller sub-group collections. As of January 2015, approximately 2 700 study members remain in active follow-up.

NSHD Biological sample collection

Biomarker data have been collected from participants at three time points, when participants were aged 53 yrs, at 60-64 yrs and most recently at 69 yrs[14]; here we present the currently available biomarker data at 53 yrs and 60-64 yrs (**Table 1**) as data from the most recent wave have not been measured by the time of this publication. At 53 yrs a number of clinical measures were collected in addition to blood (non-fasted participants, N=2756) and buccal (N=2918) samples were collected during a visit by a nurse in the participants' home; DNA was extracted from both samples (a total of 2939 study members represented). Additional aliquots of plasma and serum were stored and used in subsequent analyses following re-collection of blood samples when participants were aged 60-64 (see below). At aged 60-64, a wide-ranging collection of biological measures was conducted with additional blood (N=2143), saliva (N=1965) and overnight urine samples (N=2193) collected, either in a clinic or at home, after participants were requested to fast overnight. The collection of saliva samples only began after a pilot study, and the protocol consisted of one sample collected on the day of the clinical assessment and a further three at home. DNA was extracted from the blood samples and a number of biomarkers have been measured. Detailed description of the NSHD cohort study has been published elsewhere[15,16]. The following **Table 7** lists all biological analytes measured at 53 and 60-64 yrs.

[14] Kuh D. et al. The MRC National Survey of Health and Development reaches age 70: maintaining participation at older ages in a birth cohort study. Eur J Epidemiol. 2016, 31:1135–1147

[15] Wandsworth M. et al. Cohort Profile: The 1946 National Birth Cohort (MRC National Survey of Health and Development). Int. J. Epidemiol. 2006, 35 (1): 49-54.

[16] Kuh D. et al. Cohort Profile: Updating the cohort profile for the MRC National Survey of Health and Development: a new clinic-based data collection for aging research. Int. J. Epidemiol. (2011) 40 (1): e1-e9.

Table 7 Biomarkers available in NSHD dataset. Biomarkers highlighted in bold are unique to this dataset.

System	Biomarkers at 53 yrs	Biomarkers at 63 yrs
Metabolic	Cholesterol, Total (mmol/L) Cholesterol, High density Lipoprotein, HDL (mmol/L) Cholesterol, Low density Lipoprotein, LDL (mmol/L) Triglycerides (mmol/L) Glycated Haemoglobin, HbA1c (mmol/mol)	Adiponectin (µg/mL)***** Cholesterol, Total (mmol/L) Cholesterol, High density Lipoprotein, HDL (mmol/L) Cholesterol, Low density Lipoprotein, LDL (mmol/L) Triglycerides (mmol/L) Glucose (nmol/L) Insulin (pmol/L) Leptin (ng/mL)***** Lipoprotein[a] (mg/dL) Proinsulin (pmol/L) Glycated Haemoglobin, HbA1c (mmol/mol)
Diet & Iron Markers		Ferritin (ng/mL) Haemoglobin, Hb (g/L) **Iron** (µg/dL) Vitamin C (µmol/L) Vitamin D_3 (250HD3) (ng/mL)**†**
Inflammatory		**Anti-Thyroperoxidase, TPO (IU/mL)** Basophils ($x10^9$/L) C-Reactive Protein, CRP (mg/L) Eosinophil ($x10^9$/L) Fibrinogen (g/L) **Homocysteine concentration (µmol/L)** Interleukin-6, IL-6 (pg/mL) Tissue Plasminogen Activator, TPA (ng/mL) Von Willebrand Factor, VWF (IU/dL) Red Cell Folate, RCF (nmol/L) Lymphocytes ($x10^9$/L) Mean Corpuscular Volume, MCV Mean Corpuscular Haemoglobin, MCH **Mean Platelet Volume, MPV** Monocytes ($x10^9$/L) Neutrophils ($x10^9$/L) Platelet count ($x10^9$/L) **Platelet Distribution Width, PDW (%)** **Potassium** (mmol/L) Protein (blood), Total (g/L)***** Red Blood Cell, RBC, count (10^9/L) White Blood Cell, WBC, count (10^9/L)
Neuro-Endocrine	DHEAS (umol/L) Insulin Growth Factor 1, IGF-1 (nmol/L) Sex hormone binding globulin, SHBG (nmol/L) Testosterone (nmol/L)	Cortisol (saliva) (nmol/L) DHEAS (umol/L) Insulin Growth Factor 1, IGF-1 (nmol/L) Insulin Growth Factor 2, IGF-2 (nmol/L) Insulin Growth Factor IGF-BP3 (ng/mL) Sex hormone binding globulin, SHBG (nmol/L) Testosterone (nmol/L) Thyroid tests (T3, T4) (pmol/L)***** **Thyroid Stimulating Hormone, TSH (mU/L)**
Kidney & Liver		Calcium (mmol/L) Creatinine (blood, urine) (mmol/L) Phosphate (mmol/L) Urea (nmol/L)***** Alanine Transaminase , ALT (IU/L) Alkaline Phosphatase, ALP (IU/L) Aspartate Transaminase, AST (IU/L) Albumin (blood, urine) (mg/L) Bilirubin (mg/dL)***** **Cystatin C (mg/L)** Gamma Glutamyl Transferase, GGT (IU/L) **Glucose (urine) (dipstick) (mmol/L)** Globulin (mg/dL)***** **Ketones (urine) (dipstick) (positive/negative)** **Liver Iron Concentration, LIC (%)** Potassium (serum) (mmol/L)***** **Potassium (urine) (mmol/L)** **Protein (urine) (positive/negative)** Sodium (urine) (mmol/L)***** **Sodium (blood) (mmol/L)** **Urate** (g/L)

***Also measured in ALSPAC, but not presented in this document**
****Measured in ALSPAC for a small sample (~95 individuals)**
†Vitamin D_2 was undetectable; vitamin D_3 is representative of Total Vitamin D

NSHD Data Access

The survey data are accessible to bona fide researchers by applying through the NSHD website (http://www.nshd.mrc.ac.uk/data/data-sharing/). Research metadata (i.e. descriptions of the study and the questions asked) can be accessed via the MRC Gateway Portal (https://www.datagateway.mrc.ac.uk/), the NSHD Metadata repository (http://www.nshd.mrc.ac.uk/data/) and the online CLOSER Discovery platform (https://discovery.closer.ac.uk/).

2.6 HCS: Hertfordshire Cohort Study

The Hertfordshire Cohort Study involves men and women born in the English county of Hertfordshire between 1911 and 1939. The main aim of this study was to discover how a person's inbuilt makeup (genome), and the environment they experience during early life (in the womb and first few years of childhood), affects their health and ageing in later life.

There were 42 974 births in Hertfordshire, UK, between 1931 and 1939. Following exclusion criteria which reduced eligible records to 24 130, a total of 8 650 men and women were traced as still alive in Hertfordshire in 1998. Of these, 7 106 participants were registered in a general practice (GP) in Hertfordshire and were targeted for inclusion into the study.

HCS Biological sample collection

Permission to contact 6 099 (86%) men and women by letter was obtained from their general practitioners and 3 225 (53%) agreed to a home interview with a trained research nurse. Subsequently, 2 997 (93%) men and women attended a clinic for detailed physiological investigations where blood (after 12h overnight fast) and urine (timed overnight) were collected (**Table 1**). Additionally, participants were sent salivettes with which to collect saliva following a period of fasting[17]. A detailed description of this cohort has been published elsewhere[18].

The following **Table 8** lists the biomarkers measured from blood, saliva and urine samples.

[17] Ward A.M.V. et al. Fetal Programming of the Hypothalamic-Pituitary-Adrenal (HPA) Axis: Low Birth Weight and Central HPA Regulation. Journal of J Clin Endocrinol Metab, March 2004, 89(3): 1 227–1233

[18] Syddall H.E et al. Cohort Profile: The Hertfordshire Cohort Study. Int. J. Epidemiol. (December 2005) 34 (6): 1234-1242.

Table 8 Biomarkers available in HCS dataset. Biomarkers highlighted in bold are unique to this dataset.

System	Biomarker
Metabolic	Cholesterol, Total (mmol/L)
	Cholesterol, High Density Lipoprotein, HDL (mmol/L)
	Cholesterol, Low Density Lipoprotein, LDL (mmol/L)
	Apolipoprotein, Apo A1 (g/L)
	Apolipoprotein, Apo B (g/L)
	Lipoprotein[a] (mg/dL)
	Triglycerides (mmol/L)
	Glucosyl Galactosyl Pyridinoline (pmol/mL)
	Glucose (mmol/L)*****
	Insulin (pmol/L)*****
	Proinsulin (pmol/L)
Inflammatory	**Anticardiolipin, IgG (GPL-U/mL)**
	Anticardiolipin, IgM (MPL-U/mL)
	Antinuclear Antibody, ANA (U/mL)
	C-Reactive Protein, CRP (mg/L)
	C-Terminal cross linking telopeptide type 1 collagen, CTX-1 (blood) (ng/mL)**†**
	C-Terminal cross linking telopeptide type 2 collagen, CTX-2 (urine) (ng/mL)
	Osteocalcin (ng/mL)
	Rheumatoid Factor, RF (U/mL)
Neuro-Endocrine	Cortisol (saliva) (nmol/L)
	Cortisol (blood) (nmol/L)
	Creatinine (urine) (mg/L)

***measured from OGTT (Oral Glucose Tolerance Test)**
†CTX also measured in ALSPAC but not presented in this document

HCS Data Access

The survey data are accessible to *bona fide* researchers by contacting Professor Cyrus Cooper, Director of the MRC Lifecourse Epidemiology Unit at the University of Southampton, who can forward a collaborators' agreement.

Research metadata (i.e. descriptions of the study and the questions asked) can be accessed via the MRC Gateway Portal (https://www.datagateway.mrc.ac.uk/) and the online CLOSER Discovery platform (https://discoverycloser.ac.uk/).

3 Biomarker Glossary

3.1 Description of Tables and Plots

In what follows, we provide tables and plots for each biomarker that has been measured in more than one study within CLOSER.

Each table provides information on:

- Name of CLOSER study that measured the biomarker
- Number of valid cases available: includes all data not coded as missing, invalid, unavailable or similar.
- Laboratory method used to measure the biomarker (where available)
- Mean, standard deviation, median and interquartile range for each biomarker. For descriptive statistics, distributions have been winsorised[19] at 0.5% and 99.5%; no sample weights employed. These values are un-adjusted; thus, do not take into account participant characteristics nor medication information.

We also present crude plots for each biomarker, versus age. As the majority of biomarkers show skewed distributions (see Kernel plots, **Figs B1-B43),** we plot median values, instead of means **(Figs A1-A43)**. For those studies and sub-studies with a short age-span (SWS, HCS, and ALSPAC-pregnancy), we created equally sized age-groups and, for each biomarker, we plot its median at the lower boundary of the age-group (e.g. for an age group 30-34 yrs we plot the biomarker median at 30 yrs).

Similarly, for studies and sub-studies with a larger age-span (ALSPAC-FOM1, ALSPAC-FOF1, and UKHLS) we plot averaged data over 10yr age-groups and plot at the lower boundary. For all other studies and sub-studies (ALSPAC's focus clinics, NSHD, NCDS) we plot median values at each cohort's average age.

Kernel density plots **(Figs B1-B43)** show the large variation in biomarker distribution emphasizing the need for appropriate transformations when considering statistical analysis.

[19] Winsorising is a statistical method to replace extreme values with percentiles; we use 0.5 and 99.5 percentiles for the upper and lower end of distribution respectively.

3.2 Plot interpretation

The following plots (**FigA1-FigA43)** are not intended to represent a direct comparison of measured biomarkers between studies, but rather a general guide to each biomarker. Researchers need to take into account the following:

i. Collection of biological samples was carried out using various methods in the CLOSER studies. We highlight a number of conditions that may affect measurement throughout this catalogue. It is known that some biomarkers are sensitive to diurnal and seasonal variations and we note this where applicable. Furthermore, we note where transport of specimens and timely delivery and processing by laboratories may influence findings.

ii. Biomarkers have been measured by different laboratories worldwide, often without a golden standard, and crucially have used different technical methodologies on different instruments (see **Methods of Measurement** in following **Tables A1-A43**); thus, caution should be adopted when considering direct comparison.

iii. Biomarkers have been measured at different points in time and values may therefore be subject to secular changes in health, further militating against direct comparison.

iv. As already mentioned, these measurements are un-adjusted for population characteristics and do not take into account the medication status of participants, nor the effects of coexisting medical conditions at the time of blood/saliva/urine collection on the measured analyte.

3.3 Metabolic Biomarkers

Total Cholesterol

High Density Lipoprotein Cholesterol (HDL)

Low Density Lipoprotein Cholesterol (LDL)

Apolipoprotein A1 (Apo A1); Apolipoprotein B (Apo B)

Lipoprotein[a] (Lp[a])

Triglycerides

Fasting Glucose

Fasting Insulin

Proinsulin

Glycated Haemoglobin (HbA1c)

3.3.1 *Association of metabolic markers and the social environment*

Metabolic markers are predictive of mortality; in particular, lipids such as total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides are commonly referred to as classic cardiovascular risk factors[20],[21]. Where examined, metabolic markers are associated with social position such that disadvantaged social position is associated with adverse levels. For example, apolipoprotein A1 is raised and apolipoprotein B is lowered in participants of the Whitehall II study working in the lowest civil service employment grades[22].

However when considering lipids such as total, HDL or LDL cholesterol, this pattern is not apparent for all lipids that have been examined; total cholesterol is not socially patterned[23] while LDL-cholesterol and HDL-cholesterol have been shown to be associated with social position such that LDL-cholesterol (so-called 'bad' cholesterol) is raised and HDL-cholesterol (so-called 'good' cholesterol) is lowered in more disadvantaged groups[24]. Similar patterns are apparent for triglycerides as for lipids such that disadvantage across the lifecourse are associated with raised levels[25]. No evidence of social differences is found for lipoprotein[a] (Lp[a]).

Evidence also suggests that glycaemic traits are associated with social position[26] although these associations are not as well established as for lipids[27]. The association of pro-insulin with the social environment has not been examined, although raised levels of fasting insulin have been demonstrated in disadvantaged groups[18].

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[20] Kannel W.B. et al. Risk factors in coronary heart disease. An evaluation of several serum lipids as predictors of coronary heart disease; the Framingham study. Ann Intern Med. 1964;61:888-899.

[21] Stamler J. et al. For the Multiple Risk Factor Intervention Trial Group. Diabetes, other risk factors, and 12-year cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. Diabetes Care.1993;16:434-444.

[22] Brunner E.J. et al. Gender and employment grade differences in blood cholesterol, apolipoproteins and haemostatic factors in the Whitehall II study. Atherosclerosis. 1993 Sep;102(2):195-207.

[23] Rumble C., Pevalin D.J. Widening inequalities in the risk factors for cardiovascular disease amongst men in England between 1998 and 2006. Public Health. 2013 Jan;127(1):27-31.

[24] Craig R. and Mindell J. (eds). Health Survey for England 2006. Volume1: Cardiovascular disease and risk factors in adults.The Information Centre,Leeds,2008.

[25] Power C. et al. Lifecourse influences on health in British adults: effects of socio-economic position in childhood and adulthood. Int J Epidemiol. 2007 Jun;36(3):532-9.

[26] Moody A. et al. Social inequalities in prevalence of diagnosed and undiagnosed diabetes and impaired glucose regulation in participants in the Health Surveys for England series. BMJ Open. 2016 Feb 8;6(2):e010155.

[27] Perel P. et al. Household wealth and the metabolic syndrome in the Whitehall II study. Diabetes Care. 2006 Dec;29(12):2694-700.

Total Cholesterol, High (HDL) and Low (LDL) Density Lipoprotein cholesterol

Total cholesterol is the most common biomarker measured across all CLOSER studies that collect blood samples. HDL-cholesterol and LDL-cholesterol are measured in most of the CLOSER studies (see **Table 2**).

What are total cholesterol, HDL-cholesterol and LDL-cholesterol?

Cholesterol is a steroid that is a vital component of the lining of cells. Because it is not soluble in blood, it is transported around the body, as cargo, in cells known as lipoprotein particles. There are two kinds of lipoproteins – Apolipoprotein A and Apolipoprotein B (see section on Apo A1 and Apo B). The first of these (Apolipoprotein A) contains high-density lipoproteins (HDL), which are involved in the delivery of cholesterol to the liver for breakdown, and hence beneficial for the body. Apolipoprotein B carries low-density lipoproteins (LDL), which are taken up by blood vessels causing narrowing of arteries.

What is the clinical significance of total cholesterol, HDL-cholesterol and LDL-cholesterol?

Total cholesterol and LDL-cholesterol are risk factors for cardiovascular disease (CVD), while HDL-cholesterol is thought to be protective against it ("good cholesterol").

How is it measured?

Total cholesterol and HDL-cholesterol were measured from a blood sample using methods detailed in **Table A1 and Table A2.**
The Freidewald equation is often used to calculate LDL-cholesterol (**Table 3**); however, it is not accurate when plasma Triglyceride concentration exceeds 4.52 mmol/L (400 mg/dL).

Freidewald equation (where units = mmol/L):

$$LDL = [\text{Total cholesterol}] - [HDL + (\text{Triglycerides}/2.2)]$$

Are there clinical cut points?

Total cholesterol is categorised as healthy at 5mmol/L or less for adults.
HDL-cholesterol is categorised as healthy at 1mmol/L or above.
LDL-cholesterol is categorised as healthy at 3mmol/L or less

What should be considered in analyses?

Total cholesterol, HDL and LDL are treated with a number of lipid regulating drugs including statins and fibrates (BNF: Chapter 2.12). In women, cholesterol is high during pregnancy. Women should wait at least six weeks after the baby is born to have cholesterol measured.

Table A1. Total Cholesterol (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy†	6 533	Enzymatic colorimetric method with cholesterol esterase using Technicon RA500	-	4.9 (1.4) 4.7 3.9-5.6
	31 mths	496		4.1 (0.6) 4.1 3.7-4.6	4.2 (0.8) 4.1 3.7-4.6
	43 mths	614		3.5(0.8) 3.7 2.9-4.0	3.6(0.8) 3.5 3.0-4.1
	7 yrs	5 430		4.3 (0.6) 4.3 3.9-4.7	4.5 (0.7) 4.5 4.0-4.9
	BBS 8 yrs	870		3.8 (0.6) 3.8 3.4-4.3	4.0 (0.7) 4.0 3.5-4.5
	9 yrs	5 082		4.2 (0.6) 4.2 3.8-4.6	4.3(0.6) 4.3 3.9-4.7
	15.5 yrs	3 488		3.6 (0.6) 3.5 3.2-4.0	3.9 (0.6) 3.9 3.5-4.3
	17.5 yrs	3 287		3.6 (0.6) 3.5 3.1-3.9	3.9 (0.7) 3.9 3.5-4.4
	FOF1, Fathers	1 948		5.1 (0.9) 5.1 4.5-5.7	-
	FOM1, Mothers	4 159		-	4.9 (0.9) 4.9 4.3-5.4
UKHLS	12 895		Enzymatic methods on a Roche Modular P analyser	5.3 (1.2) 5.2 4.4-6.0	5.4 (1.1) 5.4 4.6-6.2
SWS	LP‡	1 749	Spectrophotometry on a Konelab 20 Autoanalyser Labmedics Ltd. reagent kit	-	6.8 (1.3) 6.7 5.9-7.7
NCDS	7 824		Enzymatic colorimetric CHOD-PAP method on Olympus model AU640 auto analyser	6.1 (1.1) 6.0 5.3-6.8	5.7 (1.0) 5.6 5.0-6.3
NSHD	53 yrs	2 566	Enzymatic CHOD-PAP on a Bayer DAX-72	6.0 (1.0) 6.0 5.3-6.7	6.1 (1.1) 6.0 5.4-6.8
	63 yrs	2 066		5.3 (1.1) 5.3 4.6-6.0	6.0 (1.2) 6.0 5.2-6.8
HCS	2 788		Standard enzymatic method	5.9 (1.0) 5.9 5.2-6.6	6.5 (1.2) 6.5 5.7-7.2

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**.
† Mean (SD) gestation of blood sample is 15 (8) weeks.
‡ LP: Approximate gestation age is 34 weeks.

Figure A1. Crude plot of total cholesterol (mmol/L), median, by age for each CLOSER study that measured total cholesterol.

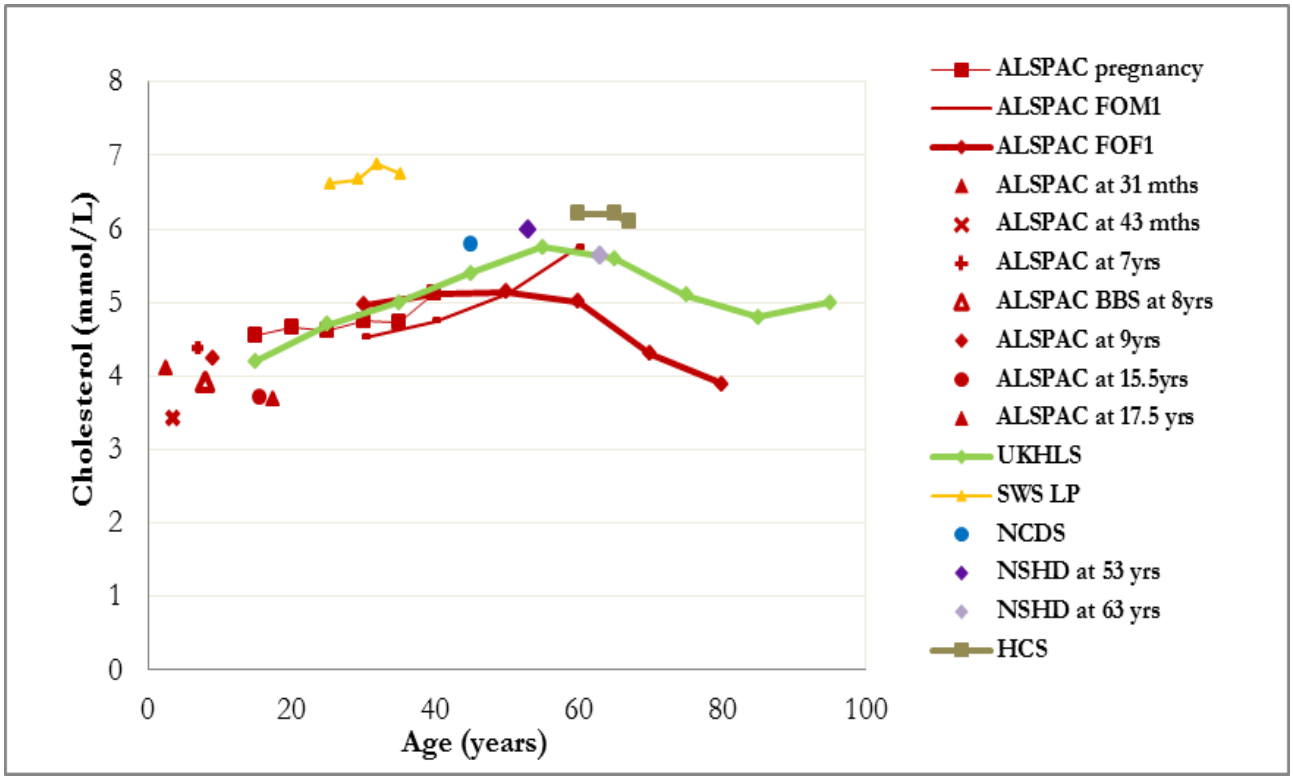
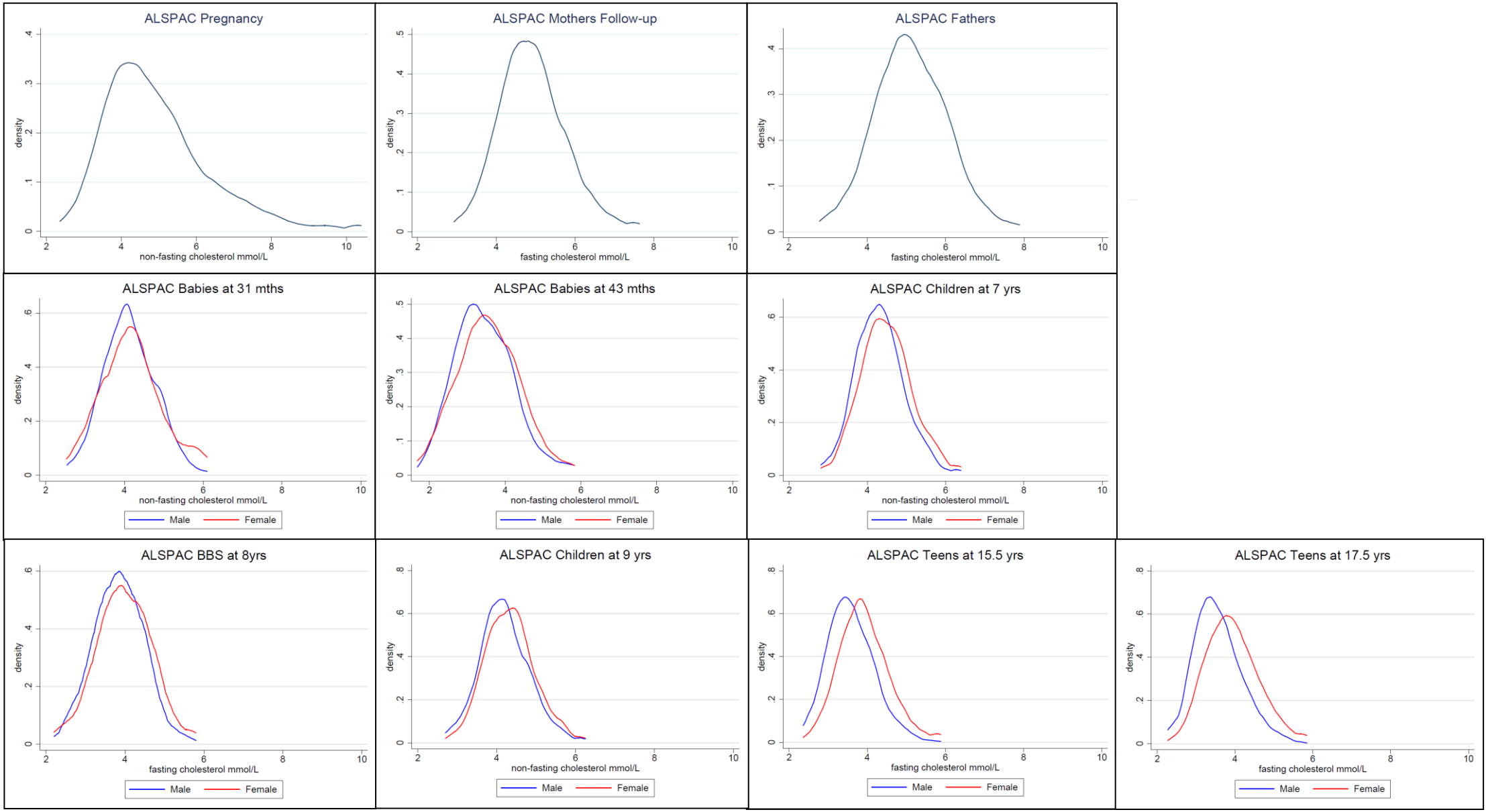


Figure B1. Distributions (Kernel density) of total cholesterol (mmol/L) by gender for each CLOSER study that measured total cholesterol. Distributions were winsorised at 0.5% and 99.5%.



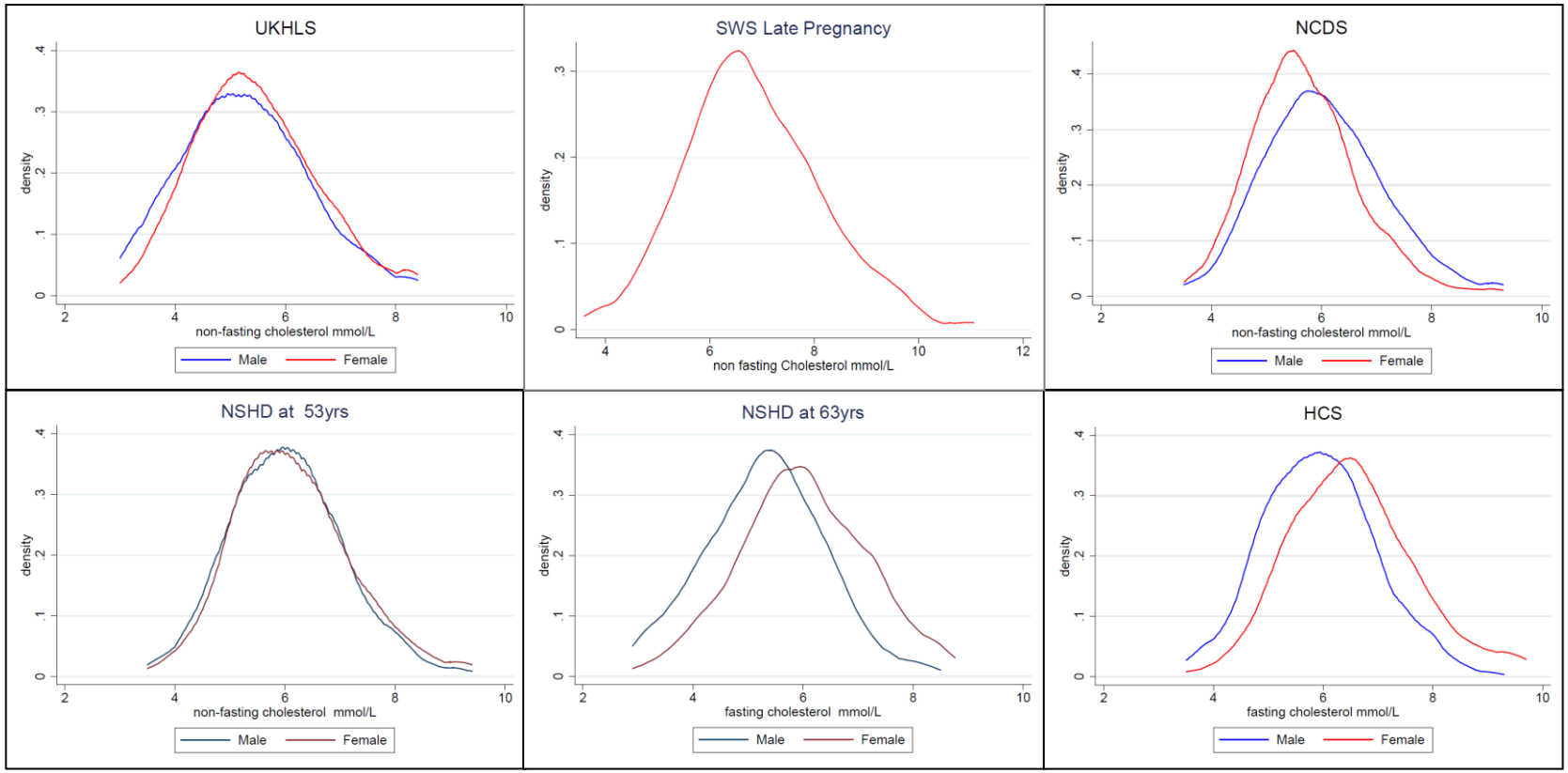

UKHLS
density
non-fasting cholesterol mmol/L
Male
Female
SWS Late Pregnancy
density
non fasting Cholesterol mmol/L
NCDS
density
non-fasting cholesterol mmol/L
Male
Female
NSHD at 53yrs
density
non-fasting cholesterol mmol/L
Male
Female
NSHD at 63yrs
density
fasting cholesterol mmol/L
Male
Female
HCS
density
fasting cholesterol mmol/L
Male
Female

Table A2. High Density Lipoprotein, HDL Cholesterol (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	31 mths	369	Colorimetric test using cholesterol esterase, after precipitation of LDL by dextran sulphate and magnesium	2.6 (0.6) 2.5 2.2-2.9	2.7 (0.7) 2.7 2.8-3.1
	43 mths	611		1.9 (0.7) 1.8 1.4-2.4	2.0 (0.8) 2.0 1.5-2.5
	7 yrs	5 430		1.5 (0.3) 1.5 1.3-1.7	1.5 (0.3) 1.5 1.3-1.7
	BBS 8 yrs	870		1.6 (0.3) 1.6 1.3-1.8	1.5 (0.3) 1.5 1.3-1.7
	9 yrs	5 082		1.4 (0.3) 1.4 1.2-1.6	1.4 (0.3) 1.3 1.2-1.6
	15.5 yrs	3 488		1.2 (0.3) 1.2 1.0-1.4	1.3 (0.3) 1.3 1.1-1.5
	17.5 yrs	3 287		1.2 (0.3) 1.2 1.0-1.3	1.3 (0.3) 1.3 1.1-1.5
	FOF1, Fathers	1 948		1.3 (0.3) 1.3 1.1-1.7	-
	FOM1, Mothers	4 159		-	1.5 (0.4) 1.4 1.2-1.7
UKHLS	12 876		Enzymatic methods on a Roche Modular P analyser	1.4 (0.4) 1.3 1.1-1.6	1.7 (0.5) 1.6 1.4-1.9
SWS	-		-	-	-
NCDS	7 808		Enzymatic colorimetric CHOD-PAP method on Olympus model AU640 auto analyser	1.4 (0.3) 1.4 1.2-1.6	1.7 (0.4) 1.7 1.4-1.9
NSHD	53 yrs	2 385	Measured using phosphotungstic Mg^{2+} on a Bayer DAX-72	1.5 (0.4) 1.4 1.2-1.7	1.8 (0.5) 1.8 1.5-2.4
	63 yrs	2 066		1.4 (0.3) 1.4 1.2-1.6	1.7 (0.4) 1.7 1.5-2.0
HCS	2 788		Measured by standard enzymatic methods on an Advia 1650 auto analyser	1.4 (0.3) 1.3 1.1-1.6	1.7 (0.4) 1.6 1.4-2.0

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A2. Crude plot of HDL cholesterol (mmol/L), median, by age for each CLOSER study that measured HDL cholesterol.

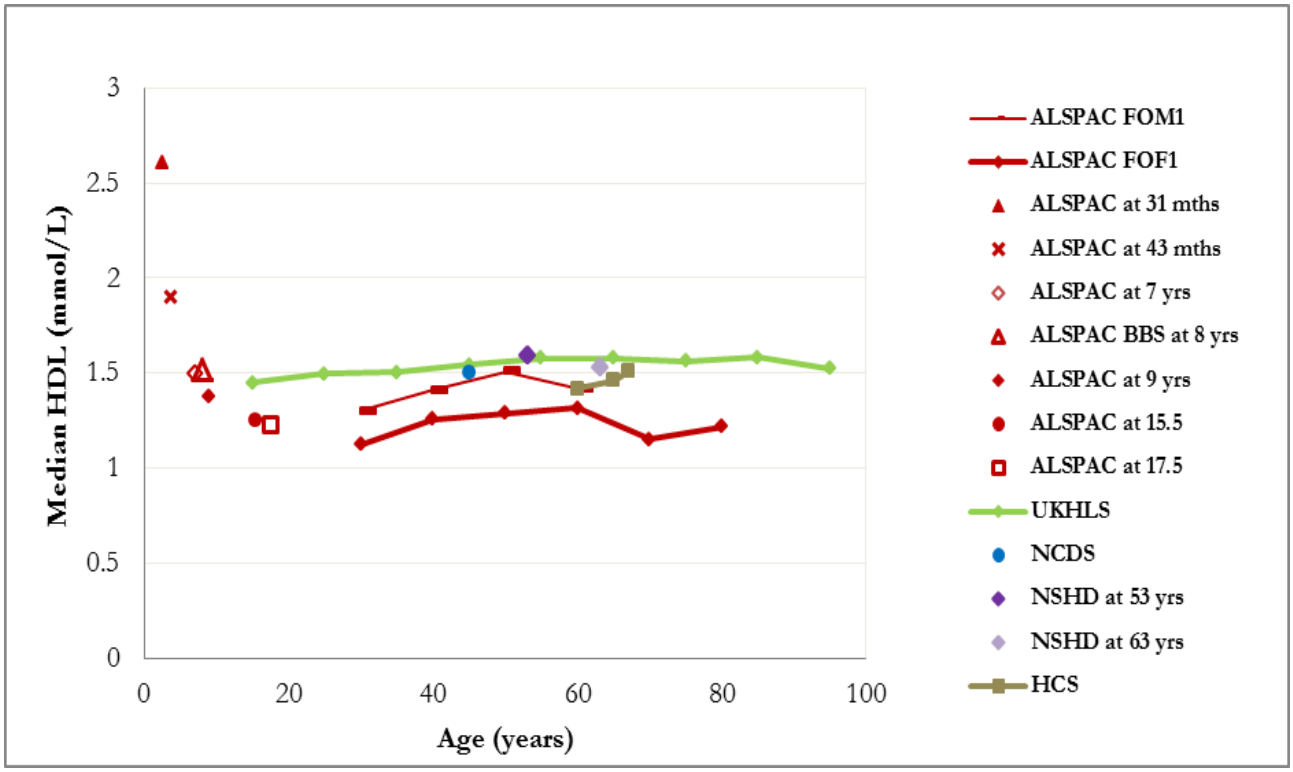
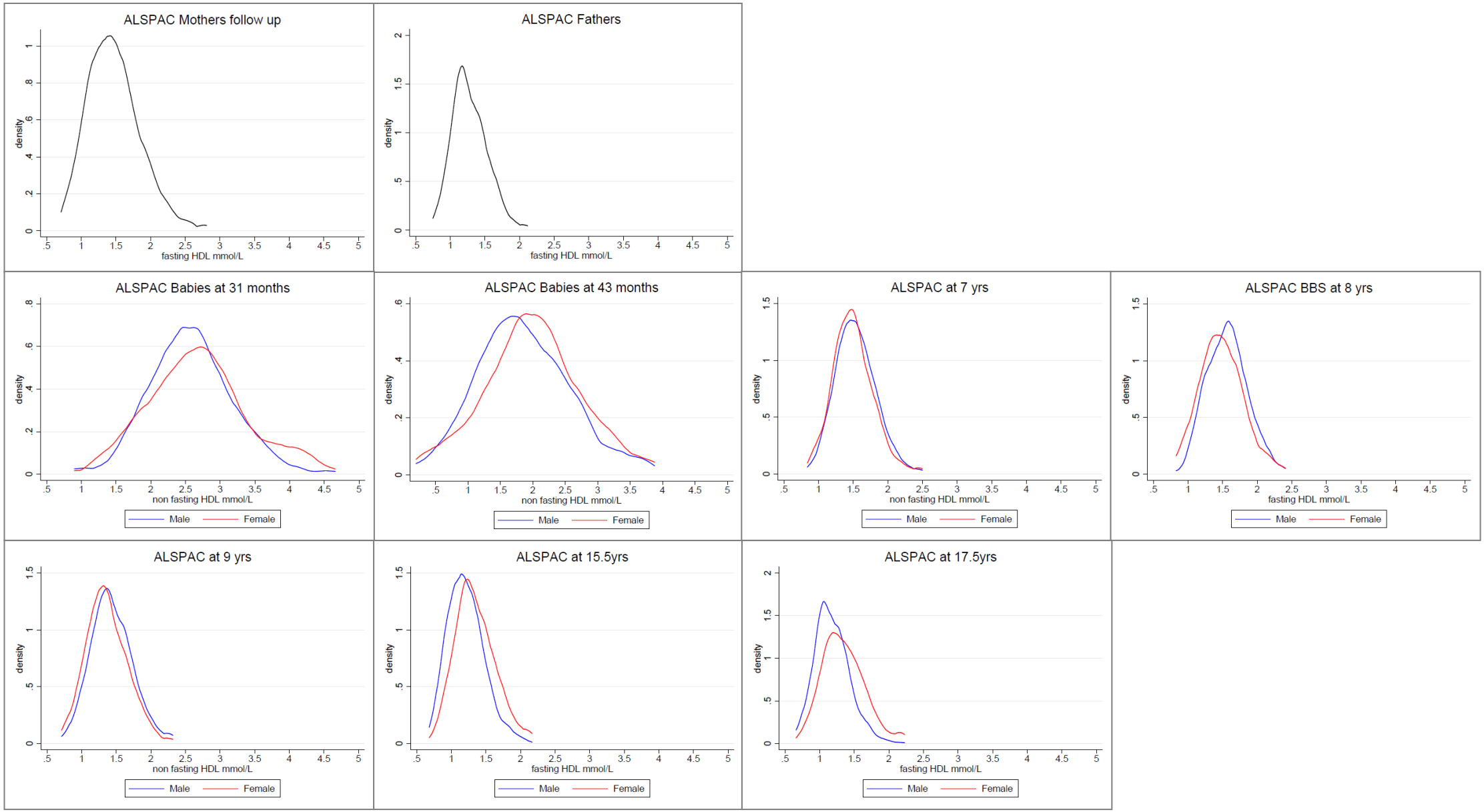


Figure B2. Distributions (Kernel density) of HDL cholesterol (mmol/L) by gender for each CLOSER study that measured HDL cholesterol. Distributions were winsorised at 0.5% and 99.5%.



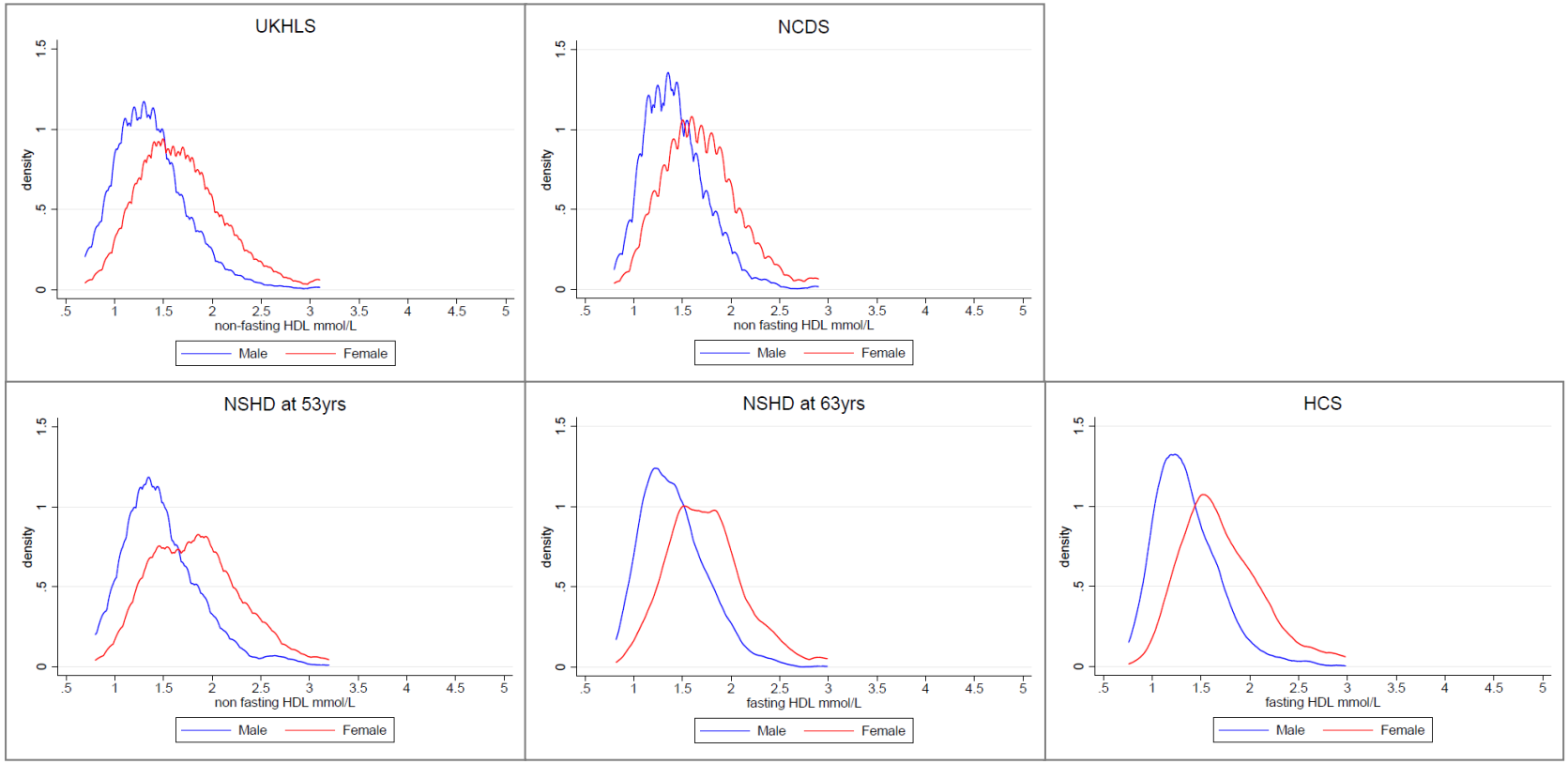
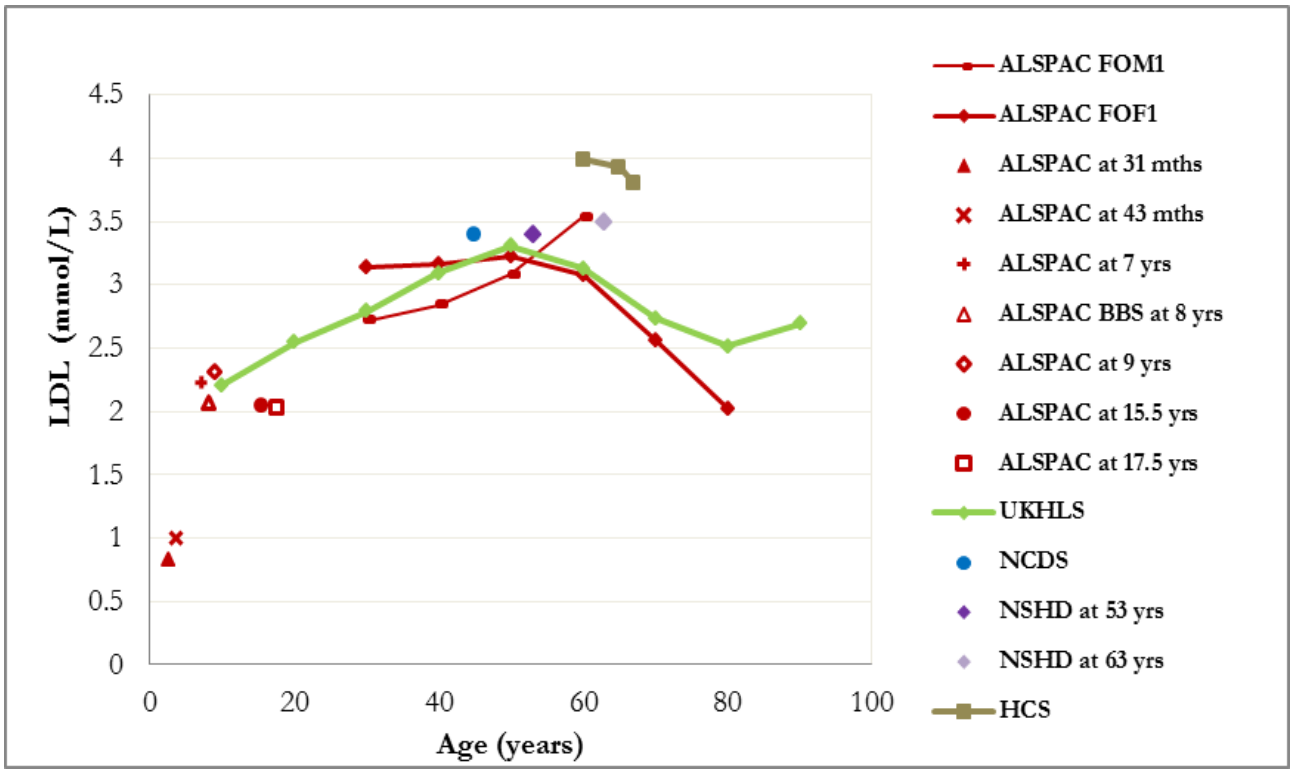

UKHLS
density
non-fasting HDL mmol/L
Male
Female
NCDS
density
non fasting HDL mmol/L
Male
Female
NSHD at 53yrs
density
non fasting HDL mmol/L
Male
Female
NSHD at 63yrs
density
fasting HDL mmol/L
Male
Female
HCS
density
fasting HDL mmol/L
Male
Female

Table A3. Low Density Lipoproteins LDL Cholesterol (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	31 mths	215	Calculated using the Freidewald formula**	0.9(0.2) 0.8 0.7-1.0	0.8(0.2) 0.8 0.7-1.0
	43 mths	615		1.0(0.3) 1.0 0.9-1.2	1.0(0.2) 1.0 0.8-1.2
	7 yrs	3 287		2.2(0.5) 2.2 1.8-2.5	2.3(0.6) 2.3 2.0-2.7
	BBS 8 yrs	870		2.0(0.5) 2.0 1.6 2.4	2.2(0.6) 2.1 1.8-2.6
	9 yrs	5 082		2.3(0.6) 2.2 1.9-2.6	2.4(0.6) 2.4 2.1-2.8
	15.5 yrs	3 488		2.0(0.5) 1.9 1.6-2.3	2.2(0.6) 2.1 1.8-2.5
	17.5 yrs	3 887		2.0(0.6) 1.9 1.6-2.3	2.2(0.6) 2.1 1.8-2.6
	FOF1, Fathers	1 370		3.2(0.8) 3.2 2.6-3.7	-
	FOM1, Mothers	4 157		-	2.3(0.8) 2.9 2.4-3.4
UKHLS	12 898		Calculated using the Freidewald formula**	3.0 (1.0) 2.9 2.2-3.6	3.0 (0.9) 3.0 2.4-3.6
SWS	-		-	-	-
NCDS	7 391		Calculated using the Friedewald formula**	3.6 (1.0) 3.5 2.9-4.1	3.3 (0.9) 3.2 2.7-3.8
NSHD	53 yrs	2 374	Calculated using the Friedewald formula**	3.5 (0.9) 3.5 2.9-4.1	3.5 (1.0) 3.4 2.8-4.1
	63 yrs	1 971		3.3 (1.0) 3.3 2.6-3.9	3.7 (1.0) 3.7 3.0-4.4
HCS	2 748		Calculated using the Friedewald formula**	3.8 (0.9) 3.8 3.2-4.4	4.1 (1.0) 4.0 3.4-4.7

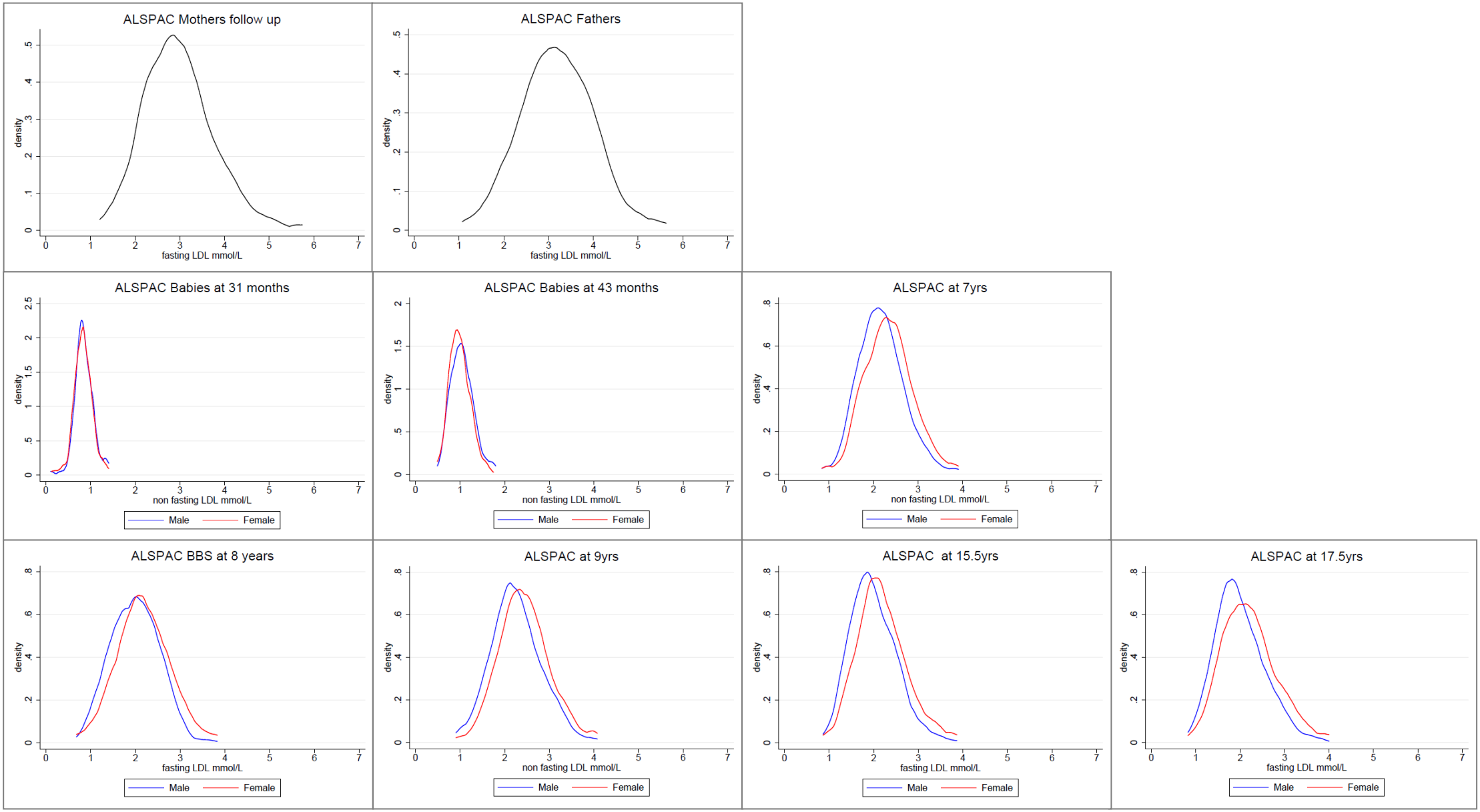
Valid cases include all data not coded as missing, invalid, unavailable or similar.
** **LDL = [Total cholesterol] – [HDL + (Triglycerides/2.2)]**

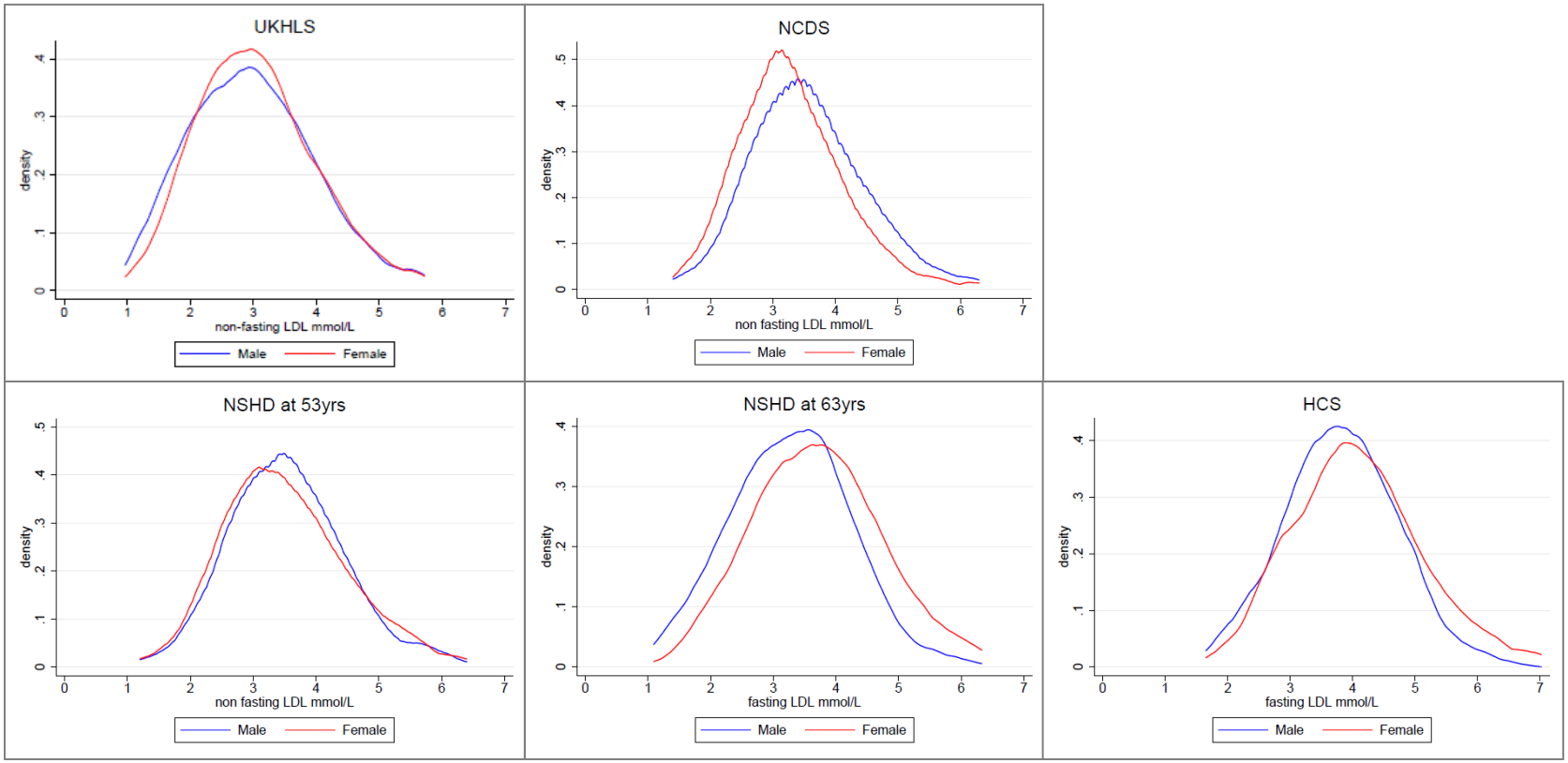
Figure A3. Crude plot of LDL cholesterol (mmol/L), median, by age for each CLOSER study that calculated LDL cholesterol according to Friedwald formula**.



** LDL = [Total cholesterol] – [HDL + (Triglycerides/2.2)]

Figure B3. Distributions (Kernel density) of LDL cholesterol (mmol/L) by gender for each CLOSER study that measured LDL cholesterol. Distributions were winsorised at 0.5% and 99.5%.




UKHLS
density
non-fasting LDL mmol/L
Male
Female
NCDS
density
non fasting LDL mmol/L
Male
Female
NSHD at 53yrs
density
non fasting LDL mmol/L
Male
Female
NSHD at 63yrs
density
fasting LDL mmol/L
Male
Female
HCS
density
fasting LDL mmol/L
Male
Female

Apolipoprotein A1 (Apo A1), Apolipoprotein B (Apo B)

Apolipoprotein A1 and Apolipoprotein B have been measured in two CLOSER studies: ALSPAC and HCS (see **Table 2**).

What is it?

Apolipoprotein A1 (Apo A1) is a protein, which is the major component of HDL-cholesterol (see next on HDL cholesterol). Apolipoprotein B (Apo B) is a protein, which is the major component of LDL-cholesterol (see next section on LDL cholesterol).

How is it measured?

A blood sample is obtained by inserting a needle into a vein in the arm. Apo A1 and Apo B are measured from plasma or serum using immunoturbidimetric methods in all studies as described in **Table A4** and **Table A5.**

What is the clinical significance of Apo A1, Apo B?

Apo A1 is involved in clearing fats and cholesterol; increased concentrations are thought to be associated with a reduced risk of cardiovascular disease. Increased levels of Apo B are associated with increased risk of cardiovascular disease. The ratio of these two correlates with the risk of cardiovascular disease.

Are there clinical cut points?

There are no clinical cut-points for Apo A1 or Apo B.

What should be considered in analyses?

Higher levels of Apo A1 are seen in women compared to men. Levels of Apo A1 can be affected by medical conditions such as chronic renal failure, coronary artery disease, uncontrolled diabetes and liver disease. Life style factors such as smoking and high fat diets also alter Apo A1 levels. Drugs such as carbamazepine, phenytoin, phenobarbital (BNF chapter 4.8.1), oestrogens (BNF chapters 6.4.1, 8.3.1), fibrates, lovastatin, pravastatin, simvastatin (BNF chapter 2.12), ethanol, niacin (BNF chapter 9.6.2), oral contraceptives (BNF 7.3), beta blockers (BNF chapter 2.4), diuretics (BNF chapter 2.2) and androgens (BNF 8.3.3) can affect Apo A1 levels.

Levels of Apo B can be affected by underlying medical conditions such as diabetes, hypo/hyper-thyroidism, kidney disease, pregnancy and diets rich in saturated fats and cholesterol as well as malnutrition. Medication intake can also alter Apo A1 levels, in particular drugs such as beta blockers, diuretics, corticosteroids (BNF chapter 6.3) and catecholamines, oestrogen (in post-menopausal women), lovastatin, simvastatin, niacin, and thyroxine (BNF chapter 6.2).

Table A4. Apolipoprotein A1, Apo A1 (g/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	9 yrs	5 082	Immunoturbidimetric assays (Hitachi/Roche)	1.4 (0.2) 1.4 1.3-1.5	1.3 (0.2) 1.3 1.2-1.5
	17.5 yrs	3 259		1.3 (0.2) 1.3 1.2-1.4	1.4 (0.2) 1.4 1.3-1.6
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	-		-	-	-
HCS	2 204		Immunoturbimetric assays using specific antibodies	1.6 (0.2) 1.5 1.4-1.7	1.8 (0.3) 1.8 1.6-2.0

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A4. Crude plot of Apolipoprotein A1 (g/L), median, by age for each CLOSER study and sub-study that measured Apo A1.

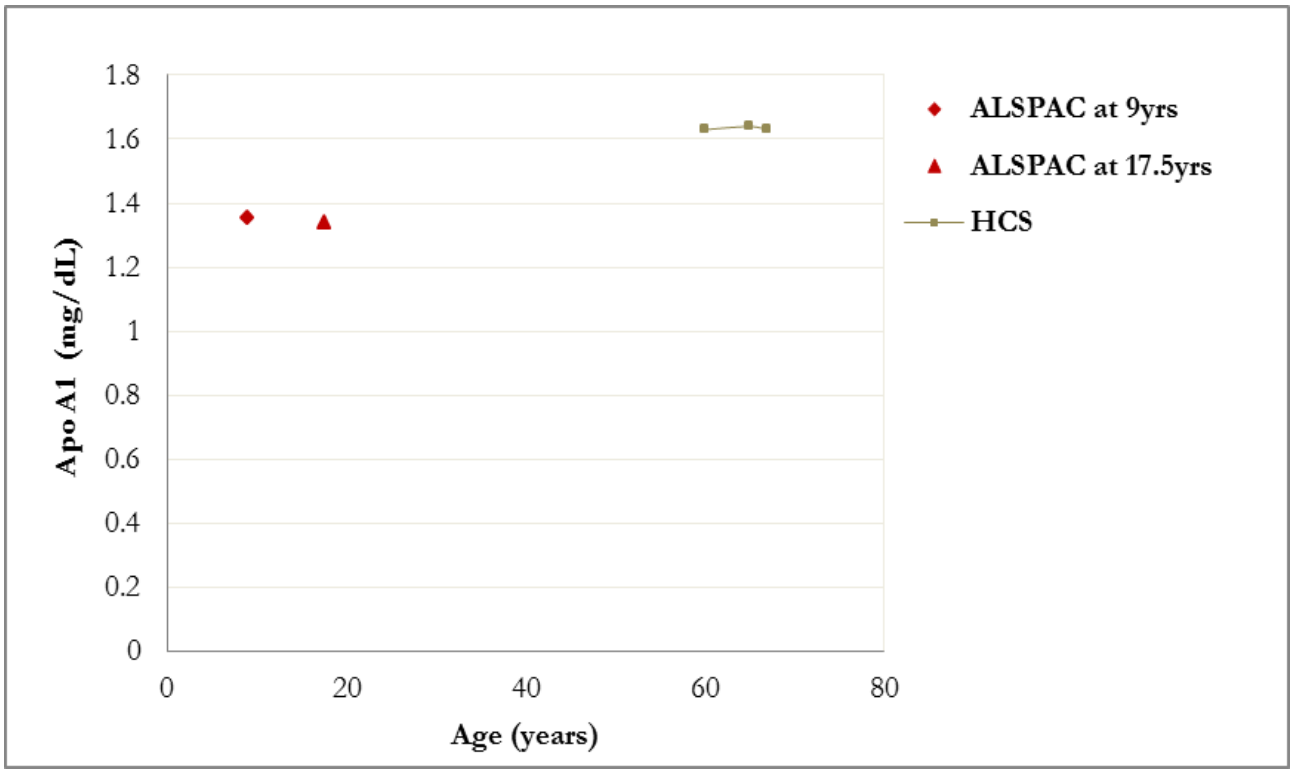


Figure B4. Distributions (Kernel density) of Apo A1 (g/L) by gender for each CLOSER study that measured Apo A1. Distributions were winsorised at 0.5% and 99.5%.

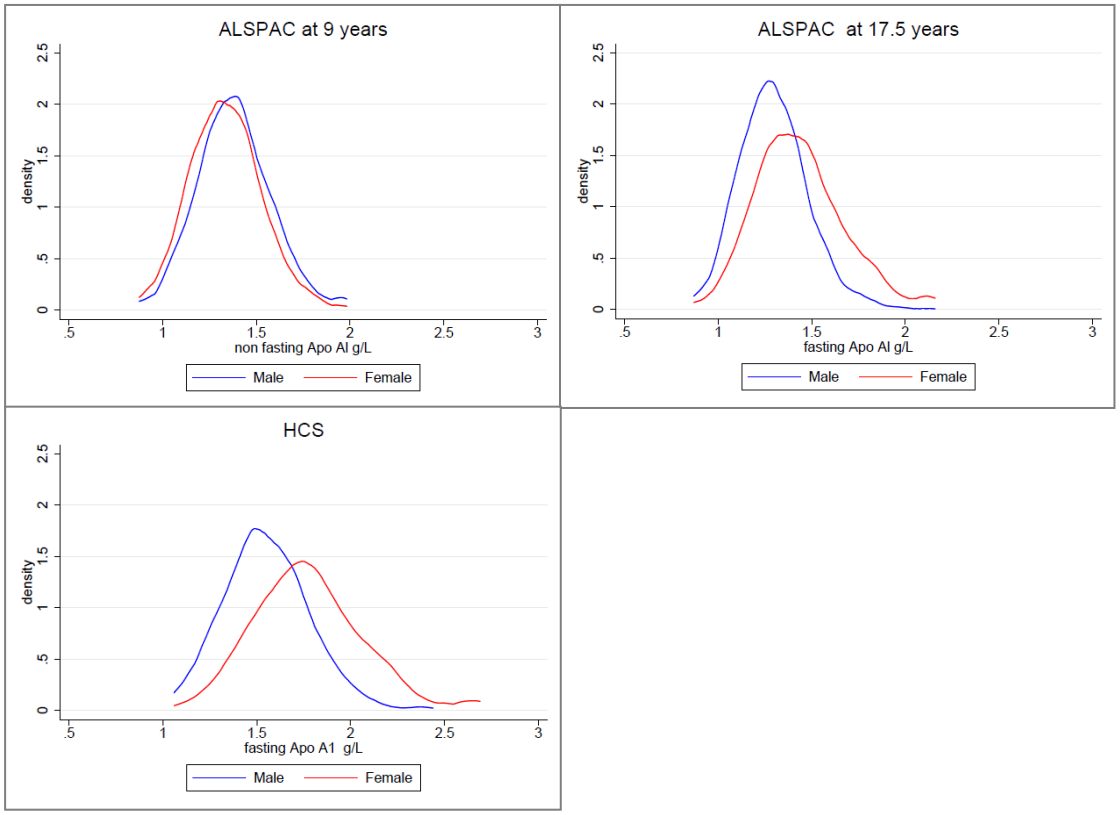


Table A5. Apolipoprotein B, Apo B (g/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	9 yrs 5 082	Immunoturbidimetric assays (Hitachi/Roche)	0.6 (0.1) 0.6 0.5-0.7	0.6 (0.1) 0.6 0.5-0.7
UKHLS	-	-	-	-
SWS	-	-	-	-
NCDS	-	-	-	-
NSHD	-	-	-	-
HCS	2 204	Immunoturbimetric assays using specific antibodies	1.2 (0.3) 1.2 1.0-1.4	1.2 (0.3) 1.2 1.0-1.4

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A5. Crude plot of Apolipoprotein B (g/L), median, by age for each CLOSER study that measured Apo B.

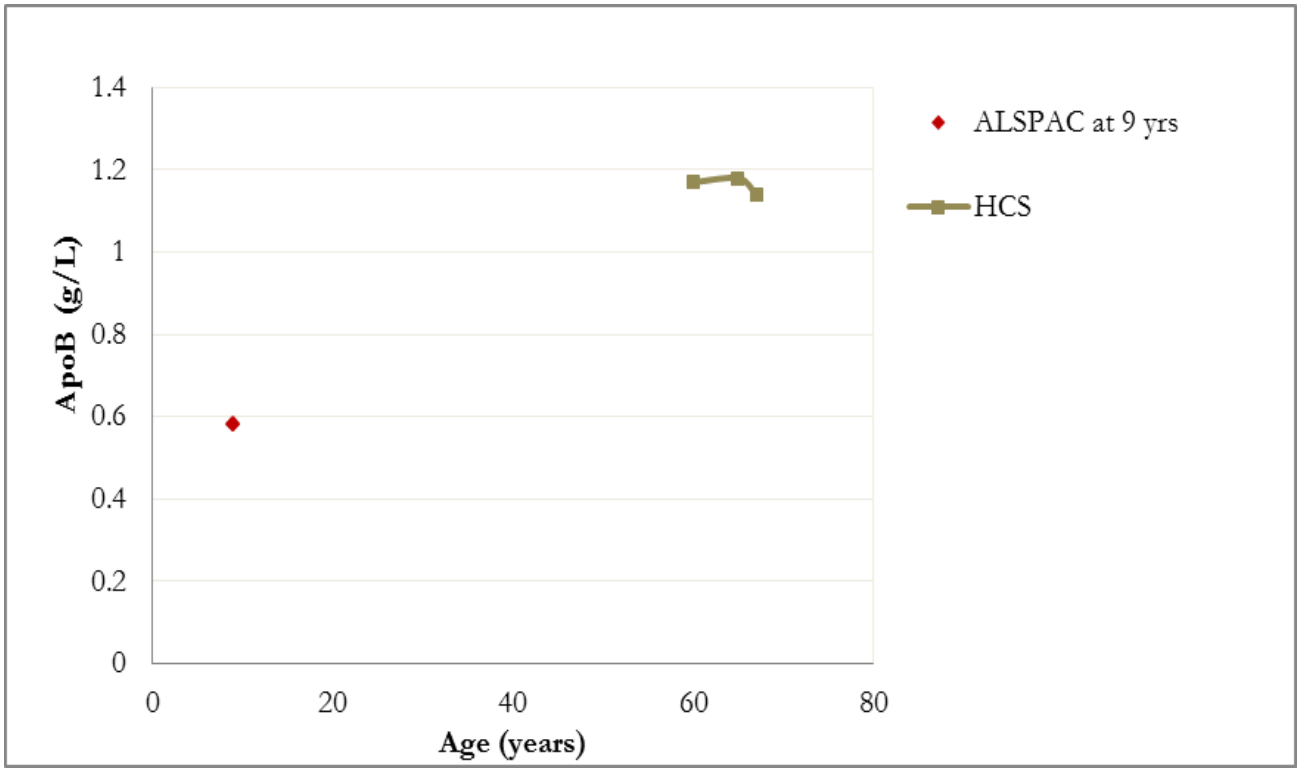
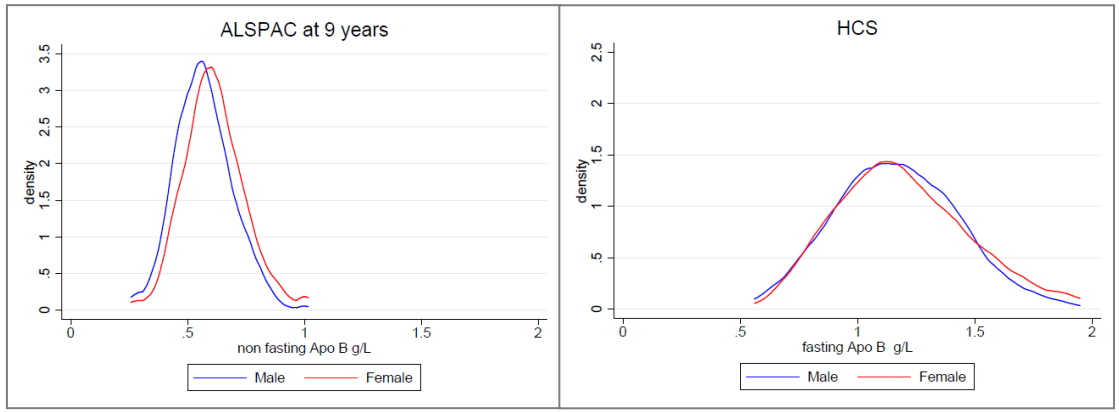


Figure B5. Distributions (Kernel density) of Apo B (g/L) by gender for each CLOSER study that measured Apo B. Distributions were winsorised at 0.5% and 99.5%.



Lipoprotein [a], Lp[a]

Lipoprotein[a] has been measured in two CLOSER studies: NSHD and HCS (see **Table 2**).

What is it?

Lipoprotein[a] is a lipoprotein formed of a lipid rich core surrounded by an apolipoprotein (ApoB-100) and an apolipoprotein A (Apo A) molecule. In the body, apolipoprotein A can interfere with the function of plasminogen, which is has clotting function to result in blood clot formation and help low density lipoprotein cholesterol (LDL) molecules bind to artery walls. This accelerates the development of plaques within the wall, which can then narrow and harden the artery. This dual action may explain the role of Lp[a] in the promotion of cardiovascular disease (CVD).

How is it measured?
Lipoprotein [a] is measured using methods described in **Table A6.**

What is the clinical significance of Lipoprotein [a]?

Lipoprotein [a] is a risk factor for heart disease especially when LDL cholesterol is also raised.

Are there clinical cut points?
Lp[a] of 30mg/dL is considered the critical threshold for cardiovascular disease (CVD).

What should be considered in analyses?

Lp[a] concentrations within the blood are genetically determined and will remain fairly constant in an individual over a lifetime[28]; population studies show ranges from <0.2 to > 200 mg/dL[29]. Concentration is not affected by diet, exercise, and other lifestyle modifications used to lower lipids within the blood. Lp[a] concentrations are slightly lower in men than in women and increase slightly in women after the menopause. The concentration of Lp[a] also varies with ethnicity: individuals of African descent can have concentrations up to 4 times higher than White Europeans[30], but they may not have a higher risk for coronary artery disease (CAD).
Conditions associated with elevated concentrations of Lp[a] include: chronic renal failure, oestrogen depletion, familial hypercholesterolemia, myocardial infarction (heart attack), premature coronary artery disease, severe hypothyroidism, stenosis of cerebral arteries, stroke, and uncontrolled diabetes. Low concentrations of Lp[a] may be seen with: alcoholism, chronic liver disease and malnutrition.

[28] Mackinnon L.T., Hubinger L.M. Effects of exercise on lipoprotein[a]. Sports Med. 1999 Jul;28(1):11-24.
[29] Dumitrescu L. et al. Variation in LPA Is Associated with Lp[a] Levels in Three Populations from the Third National Health and Nutrition Examination Survey PLoS One. 2011; 6(1): e16604. doi:10.1371/journal.pone.0016604
[30] Virani S.S. et al. Associations between lipoprotein[a] levels and cardiovascular outcomes in black and Caucasian subjects: the Atherosclerosis Risk in Communities (ARIC) Study. Circulation 2012;125:241–249.

Table A6. Lipoprotein[a], Lp[a] (mg/dL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	-		-	-	-
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	2 055	Automated analyser (Roche)	25.7 (33.5) 10.1 3.6-36.4	29.9 (36.2) 13.5 4.5-45.4
HCS	2 199		Immunoturbimetric assays using specific antibodies	21.1 (24.9) 9.7 4.0-31.0	23.5 (27.3) 12.0 5.1-31.4

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**.

Figure A6. Crude plot of lipoprotein[a] (mg/dL), median, by age for each CLOSER study that measured lipoprotein[a].

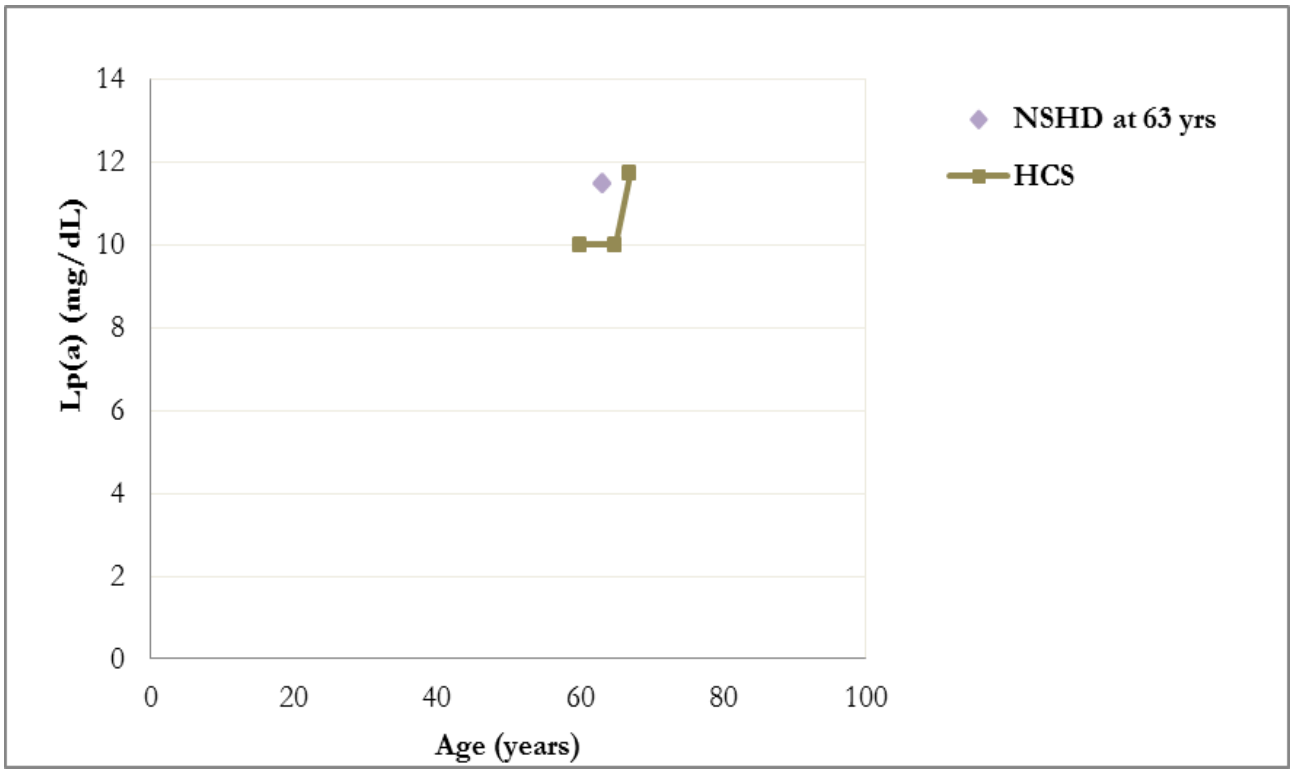
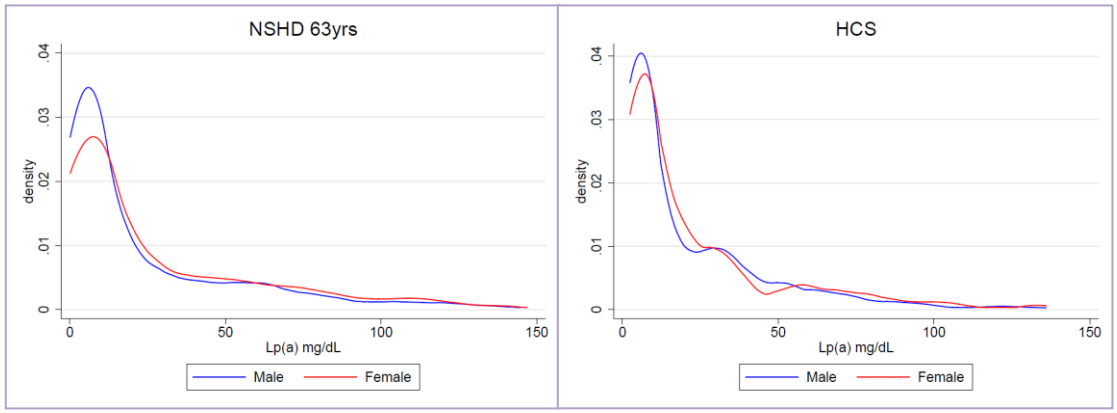


Figure B6. Distributions (Kernel density) of Lipoprotein [a] (mg/dL) by gender for each CLOSER study that measured Lp[a]. Distributions were winsorised at 0.5% and 99.5%.



Triglycerides

Triglycerides are measured in all CLOSER studies, except in SWS (see **Table 2**).

What is it?

Triglycerides are fats that are transported in the blood in conjunction with Apolipoprotein B complexes described above.

What is the clinical significance of Triglycerides?

Triglycerides levels are predictive of coronary heart disease[12].

How is it measured?

Triglycerides can be measured from whole, plasma, or serum blood samples. Triglycerides are measured using enzymatic methods in all studies as listed in **Table A7**. New consensus indicates that participants are not required to fast in order to obtain an accurate lipid profile.[31] Plasma levels of triglycerides are influenced by recent food intake, however, evidence suggests that these changes are small and do not obscure associations with cardiovascular disease[32].

Are there clinical cut points?

The desirable non-fasting triglyceride level is <2mmol/L[33].
If triglyceride concentration is very high (e.g. at least 10-15mmol/L), this indicates a risk of pancreatitis.

What should be considered in analyses?

There are many factors that can cause high levels of triglycerides such as a high fat or high sugar diet, high intake of alcohol, obesity and diabetes. Triglyceride levels are influenced by drugs, such as statins, fibrates and nicotinic acid (BNF Chapter 2.12).

[31] Nordestgaard B.G. et al. Fasting is not routinely required for determination of a lipid profile: clinical and laboratory implications including flagging at desirable concentration cut-points—a joint consensus statement from the European Atherosclerosis Society and European Federation of Clinical Chemistry and Laboratory Medicine. European Heart Journal (2016) 37, 1944–1958

[32] Nordestdgaard B.G., Varbo A. Triglycerides and cardiovascular disease. Lancet 2014;384: 626–635

[33] Kolovou, G.D. et al. Assessment and Clinical Relevance of Non-Fasting and Postprandial Triglycerides: An Expert Panel Statement. Current Vascular Pharmacology 2011: 9(3); 258-270(13)

Table A7. Triglycerides (mmol/L): Study, valid cases, laboratory method of, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	31 mths	497	Enzymatic colorimetric test	1.5 (0.7) 1.3 1.0-1.8	1.4 (0.6) 1.2 1.0-1.7
	43 mths	616		1.2 (0.6) 1.1 0.8-1.5	1.2 (0.6) 1.1 0.8-1.6
	7 yrs	5 430		1.0 (0.5) 0.9 0.7-1.2	1.1 (0.5) 1.0 0.7-1.3
	9 yrs	5 082		1.1 (0.5) 1.0 0.7-1.4	1.2 (0.5) 1.0 0.8-1.4
	15.5 yrs	3 488		0.8 (0.4) 0.7 0.6-1.0	0.8 (0.3) 0.8 0.6-1.0
	17.5 yrs	3 287		0.8 (0.4) 0.7 0.6-1.0	0.8 (0.3) 0.8 0.6-1.0
	FOF1, Fathers	1 948		1.4 (0.7) 1.3 0.9-1.8	-
	FOM1, Mothers	4 159		-	1.0 (0.5) 0.9 0.7-1.2
UKHLS	12 898		Enzymatic method on a Roche P module analyser	2.8 (5.1) 1.3 0.7-2.8	3.3 (5.4) 1.6 0.7-3.6
SWS	-		-	-	-
NCDS	7 799		Enzymatic colorimetric GPO-PAP method on Olympus model AU640 auto analyser	2.5 (1.6) 2.1 1.4-3.0	1.59 (1.0) 1.3 0.9-2.0
NSHD	53 yrs	2 562	Enzymatic method, using a glycerol/ kinase POD-linked reaction of glycerol liberated from triglycerides on a Bayer DAX-72	2.4 (1.6) 2.0 1.4-3.0	1.8 (1.2) 1.5 1.1-2.2
	63 yrs	1 983		1.4 (0.8) 1.2 0.9-1.8	1.2 (0.7) 1.1 0.8-1.5
HCS	2 788		Enzymatic method on an Advia 1650 auto analyser	1.6 (0.9) 1.4 1.0-2.0	1.6 (0.8) 1.5 1.1-2.0

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A7. Crude plot of triglycerides (mmol/L), median, by age for each CLOSER study that measured triglycerides.

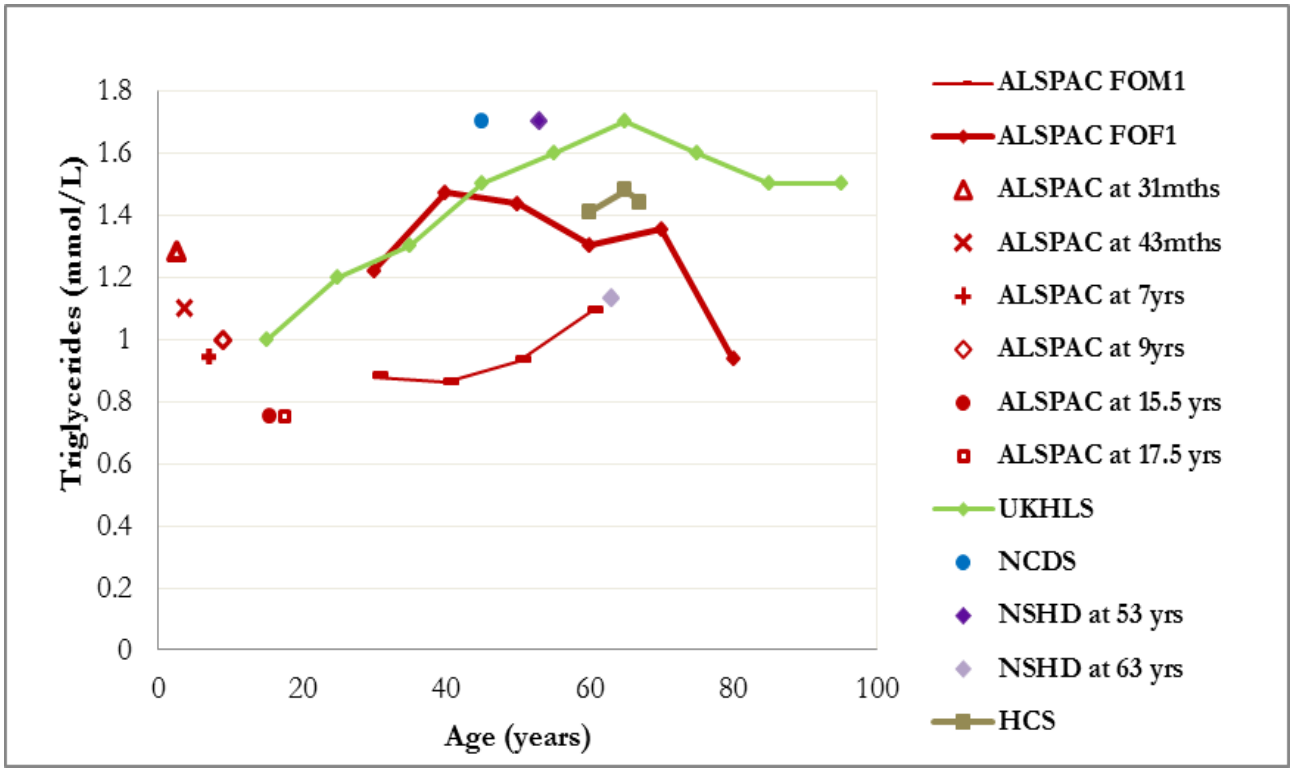
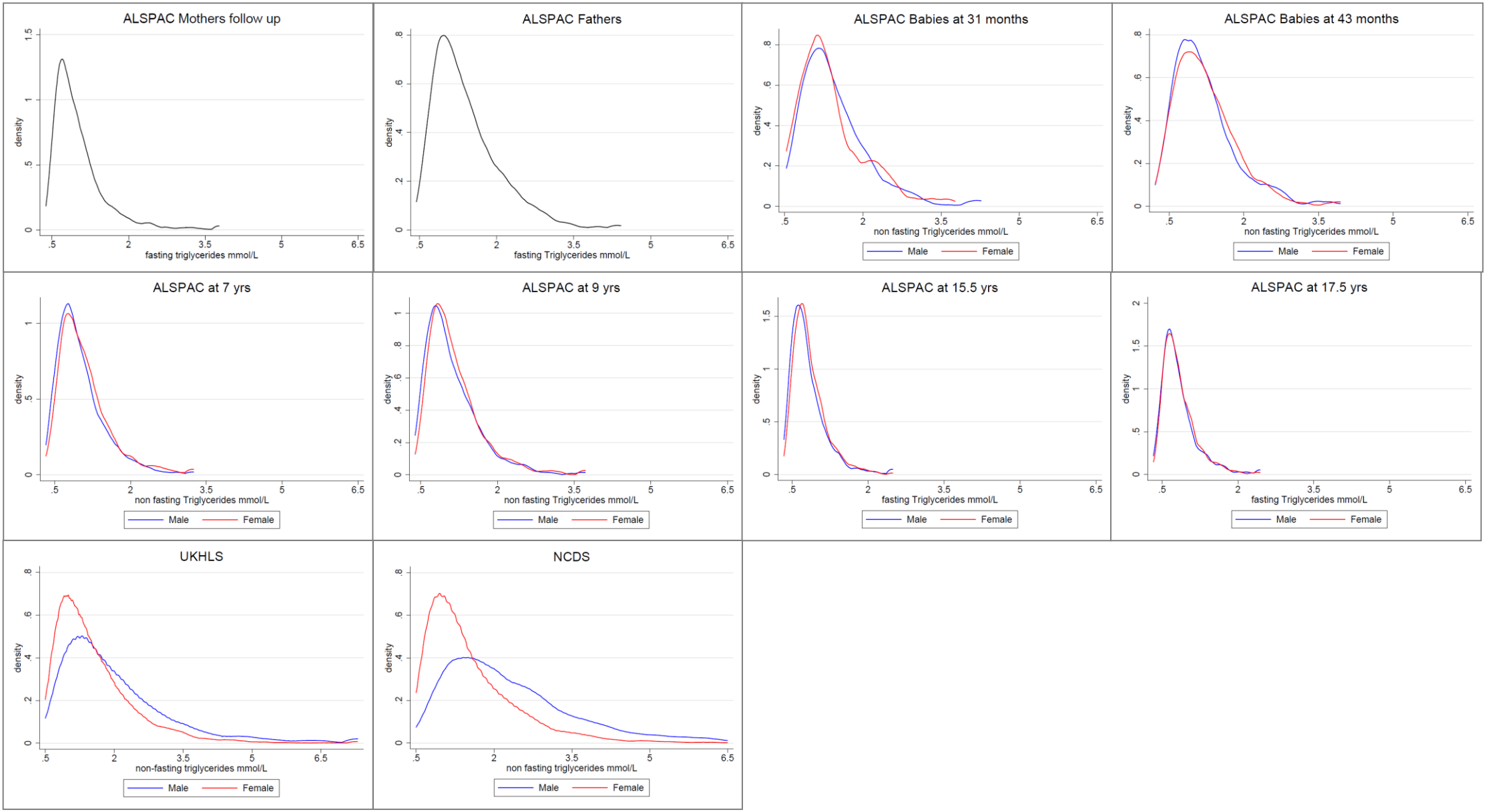
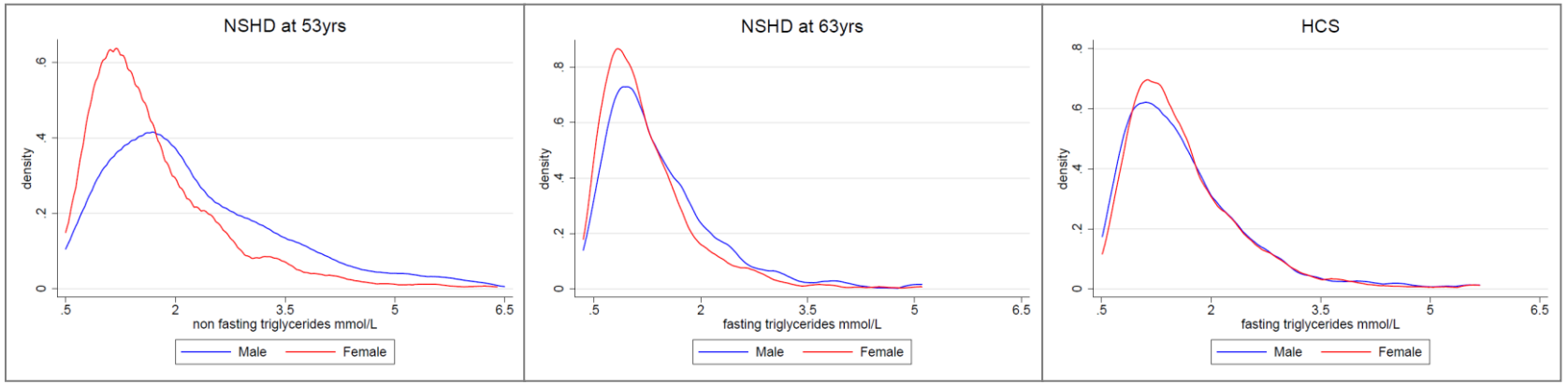


Figure B7 Distributions (Kernel density) of triglycerides (mmol/L) by gender for each CLOSER study that measured triglycerides. Distributions were winsorised at 0.5% and 99.5%.




NSHD at 53yrs
density
0
.2
.4
.6
.5
2
3.5
5
6.5
non fasting triglycerides mmol/L
Male
Female
NSHD at 63yrs
density
0
.2
.4
.6
.8
.5
2
3.5
5
6.5
fasting triglycerides mmol/L
Male
Female
HCS
density
0
.2
.4
.6
.8
.5
2
3.5
5
6.5
fasting triglycerides mmol/L
Male
Female

Fasting Glucose

Glucose has been measured in three CLOSER studies: ALSPAC, NSHD and HCS (see **Table 2**).

What is it?

Glucose is a type of sugar that is transported through the bloodstream to supply energy to all the cells in our bodies; it is primarily made from the dietary intake of carbohydrates.

How is it measured?

Glucose is measured using methods described in **Table A8.** In general, it is recommended that participants fast (nothing to eat or drink except water) for at least 8 hours (generally 8-10 hours) before a blood glucose test is performed. For the diagnosis of diabetes, fasting glucose levels are often assessed in the morning. For people with diabetes, glucose levels are often checked both while fasting and after meals to provide the best control of diabetes

What is the clinical significance of Glucose?

The human body regulates blood glucose levels so that they are neither too high nor too low; maintaining a condition of stability or equilibrium in the blood's internal environment (homeostasis) is necessary for our bodies to function.

Are there clinical cut points?

Levels of 7mmol/L, or more, probably indicate diabetes.

What should be considered in analyses?

High levels of glucose (≥7mmol/L) indicate diabetes but many other diseases and conditions can also cause raised glucose concentrations in the bloodstream; these include acromegaly, acute stress (response to trauma, heart attack, and stroke for instance), long-term kidney disease, Cushing's syndrome, hyperthyroidism, pancreatic cancer and pancreatitis. Drugs that can alter glucose level include corticosteroids (BNF 6.3), tricyclic antidepressants (BNF chapter 4.3.1), diuretics (BNF chapter 2.2), oestrogens (birth control pills and hormone replacement therapy (HRT), lithium (BNF chapter 4.7.4), phenytoin (BNF chapter 4.8.1) and aspirin (BNF chapter 4.7.1).

Low levels of glucose can be caused by conditions such as adrenal disease (Addison's disease), extensive liver disease, hypopituitarism, hypothyroidism, and by alcohol intake, starvation and drugs such as paracetamol (BNF chapter 4.7.1) and anabolic steroids (BNF chapter 6.4.3).

Table A8. Fasting Glucose (mmol/L) Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	BBS 8 yrs	894	Glucose oxidase assay	5.0 (0.4) 5.0 4.8-5.2	4.9 (0.4) 4.8 4.7-5.1
	15.5 yrs	3 488		5.3 (0.4) 5.3 5.1-5.5	5.1 (0.4) 5.1 4.9-5.3
	17.5 yrs	3 287		5.1 (0.4) 5.1 4.9-5.4	4.9 (0.4) 4.9 4.7-5.1
	FOF1, Fathers	1 948		5.7 (1.1) 5.5 5.2-5.8	-
	FOM1, Mothers	4 159		-	5.3 (0.8) 5.2 4.9-5.4
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	2 013	Enzymatic assay using hexokinase coupled to glucose-6-phosphate dehydrogenase	5.9 (1.3) 5.6 5.3-6.2	5.6 (1.2) 5.4 5.0-5.8
HCS	2 781		Automated hexokinase method	6.0 (0.9) 5.9 5.5-6.3	5.8 (0.7) 5.7 5.4-6.1

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A8. Crude plot of fasting glucose (mmol/L), median, by age for each CLOSER study that measured glucose.

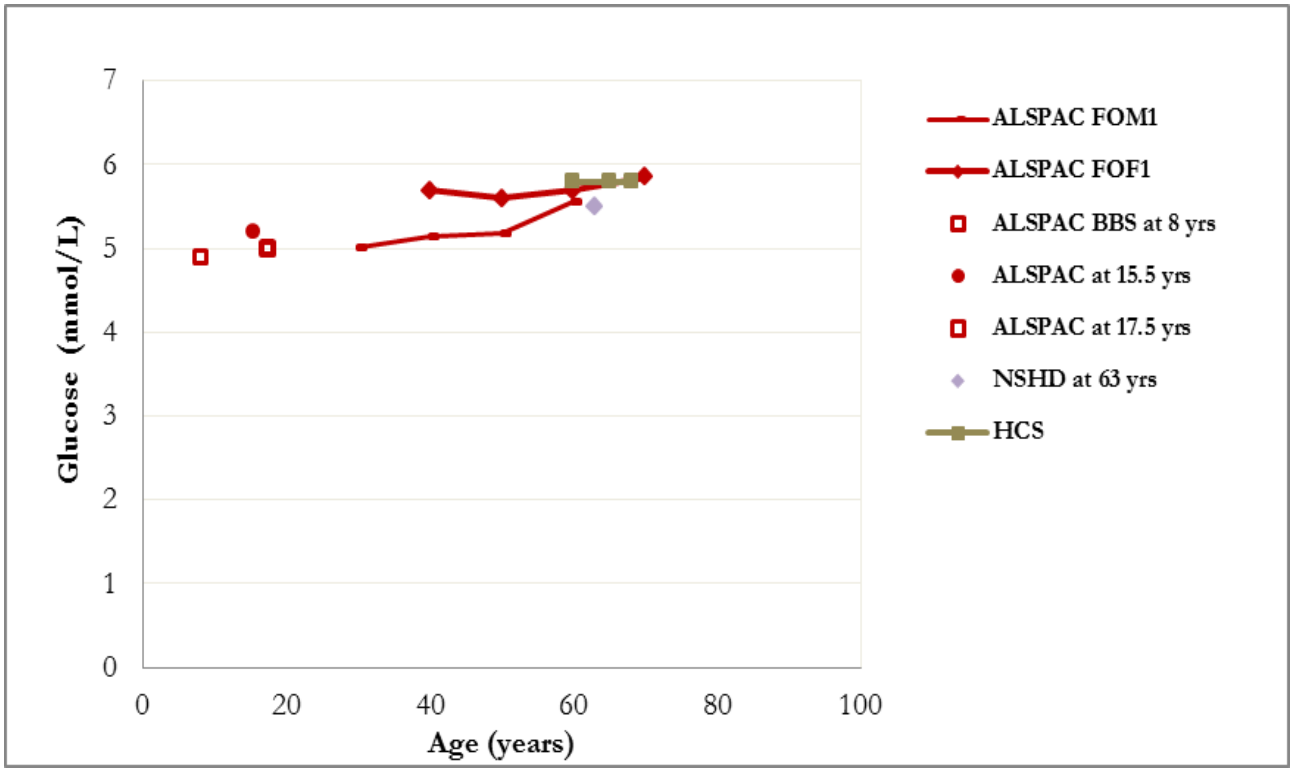
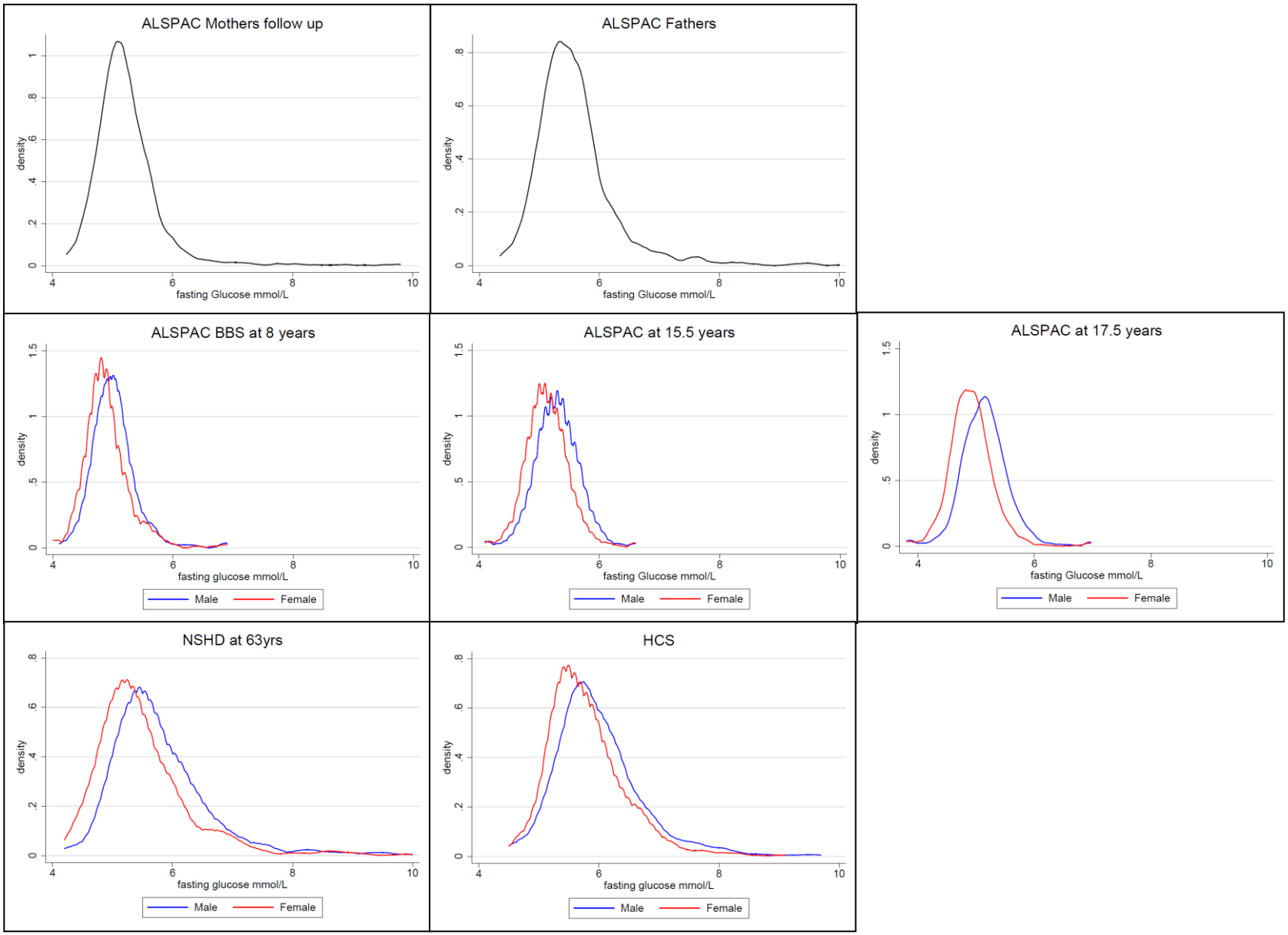


Figure B8. Distributions (Kernel density) of fasting glucose (mmol/L) by gender for each CLOSER study that measured fasting glucose. Distributions were winsorised at 0.5% and 99.5%.



Fasting Insulin

Fasting insulin has been measured in three CLOSER studies: ALSPAC, NSHD and HCS (see **Table 2**).

What is it?

Insulin is a hormone that is produced and stored in the beta cells of the pancreas. Insulin plays a key role in maintaining blood glucose at the right level.

What is the clinical significance of insulin?

Too little insulin leads to a certain type of diabetes. High levels of insulin can harm your health by leading to hypoglycaemia, or low blood sugar. Insulin levels are also sometimes used in conjunction with the glucose tolerance test (GTT), measured at pre-established time intervals to evaluate insulin resistance.

How is it measured?

A blood sample is obtained by inserting a needle into a vein in the arm. Insulin is measured using methods described in **Table A9.** Insulin levels should be measured from participants that have fasted.

Insulin was measured in international units, μIU/mL. for participants in ALSPAC BBS at 8 yrs and 15.5 yrs, We used the standard conversion factor of 6.945 to convert to SI units, pmol/L.

Are there clinical cut points?

There are no standardized clinical cut points for insulin and results are not always comparable between laboratories.

What should be considered in analyses?

Raised insulin concentrations may be seen with conditions such as acromegaly, Cushing's syndrome, fructose or galactose intolerance, insulinomas, obesity and insulin resistance, such as appears in early type 2 diabetes. Analytical interference, such as from exogenous insulin administration or insulin autoantibodies will also raise insulin levels. Decreased insulin concentrations are seen with diabetes and hypopituitarism.
Drugs such as corticosteroids (BNF chapter 6.3), levodopa (BNF chapter 4.9.1, for treatment of Parkinson’s disease), diabetes medications and oral contraceptives (BNF chapter 7.3) will influence insulin concentrations.

Table A9. Fasting Insulin (pmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	BBS 8 yrs	894	Ultrasensitive ELISA automated microparticle enzyme immunoassay that does not cross-react with proinsulin	38.0 (0.9) 24.6 17.4-42.6	42.9 (0.9) 31.8 21.0-50.4
	15.5 yrs	3 484		57.4 (0.5) 49.3 6.4-67.5	67.5 (0.6) 59.3 44.6-79.5
	17.5 yrs	3 233		45.9 (0.4) 36.5 26.7-51.9	52.4 (0.4) 44.5 32.7-59.9
	FOM1, Mothers	3 299		-	35.8 (0.3) 27.8 19.5-41.1
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	1 038	DELFIA two-site fluoroimmunometric assay	58.2 (36.9) 47.0 31.0-77.0	47.5 (29.1) 41.0 28.0-59.0
HCS	2 723		Two-site immuno-metric assays	90.3 (66.2) 71.8 46.2-110.1	83.2 (56.3) 66.2 45.5-106.3

***Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A9. Crude plot of fasting insulin (pmol/L), median by age for each CLOSER study that measured insulin.

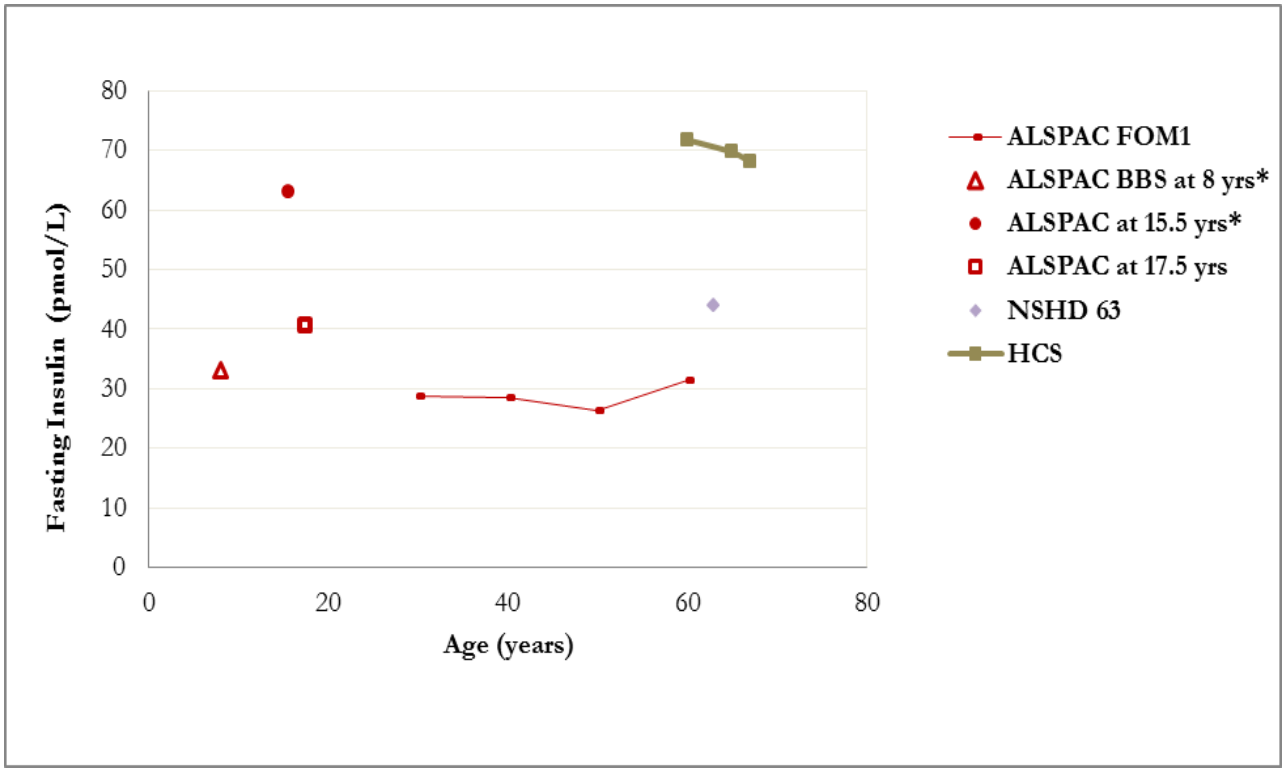
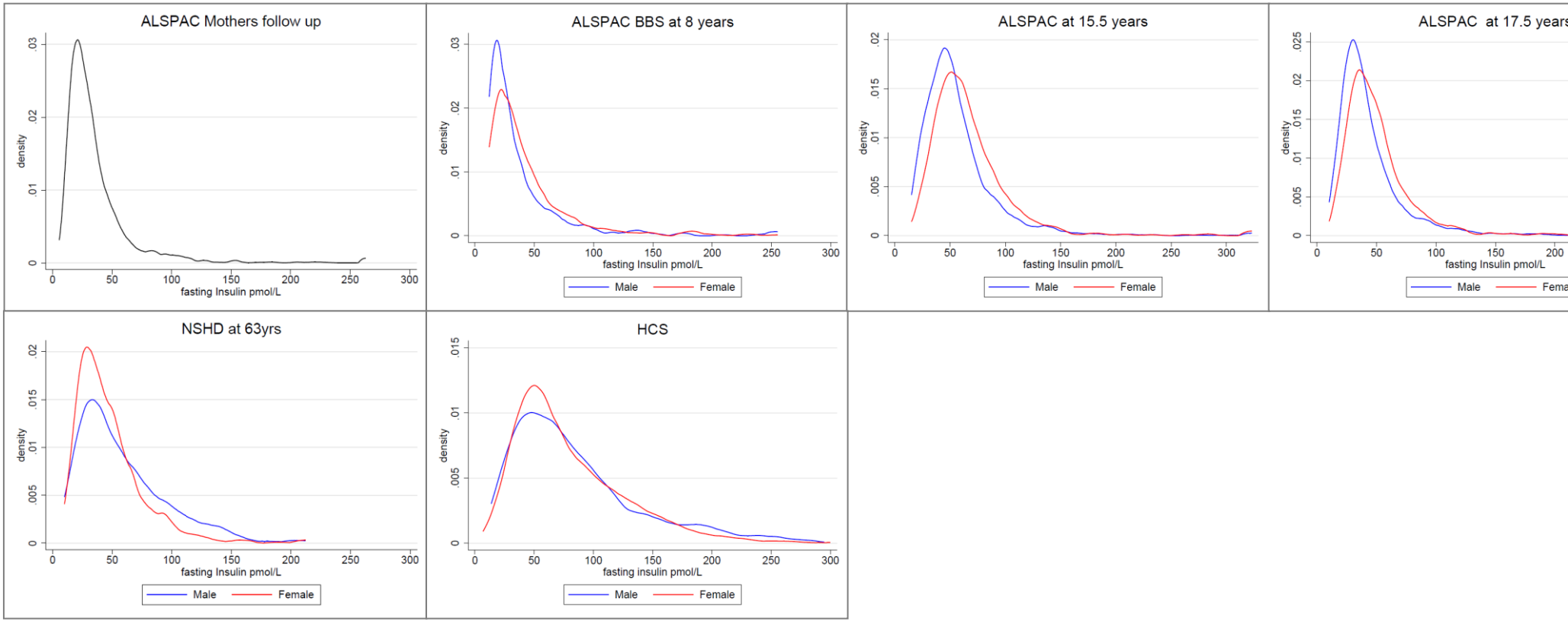


Figure B9. Distributions (Kernel density) of fasting insulin (pmol/L) by gender for each CLOSER study that measured insulin. Distributions were winsorised at 0.5% and 99.5%.



Proinsulin

Proinsulin has been measured in three CLOSER studies: ALSPAC, NSHD and HCS (see **Table 2**).

What is it?

Proinsulin is a precursor to insulin and is converted into insulin in the pancreas. Proinsulin levels represent a proportion of the insulin-like factors in serum.

What is the clinical significance of proinsulin?

High levels of proinsulin may reflect incipient insulin resistance and have also been linked to heart and artery disease.

How is it measured?

Proinsulin is measured as described in **Table A10.** Proinsulin levels should be measured from participants that have fasted.

Are there clinical cut points?

There are no standardized clinical cut points for proinsulin and results are not always comparable between laboratories.

What should be considered in analyses?

Proinsulin levels are sensitive to fasting status.

Table A10. Proinsulin (pmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	FOM1, Mothers	3 299	ELISA (Mercodia)	-	8.4 (8.0) 5.9 4.6-8.6
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	1 798	Enzyme-linked immunosorbent assay (ELISA)	13.4 (12.5) 9.2 5.9-16.2	10.1 (9.7) 7.2 5.1-11.1
HCS	1 629		Two-site immune-metric assays	15.7 (13.0) 11.7 7.6-18.9	14.4 (10.5) 11.1 7.6-16.9

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A10. Crude plot of proinsulin (pmol/L), median, by age for each CLOSER study that measured proinsulin.

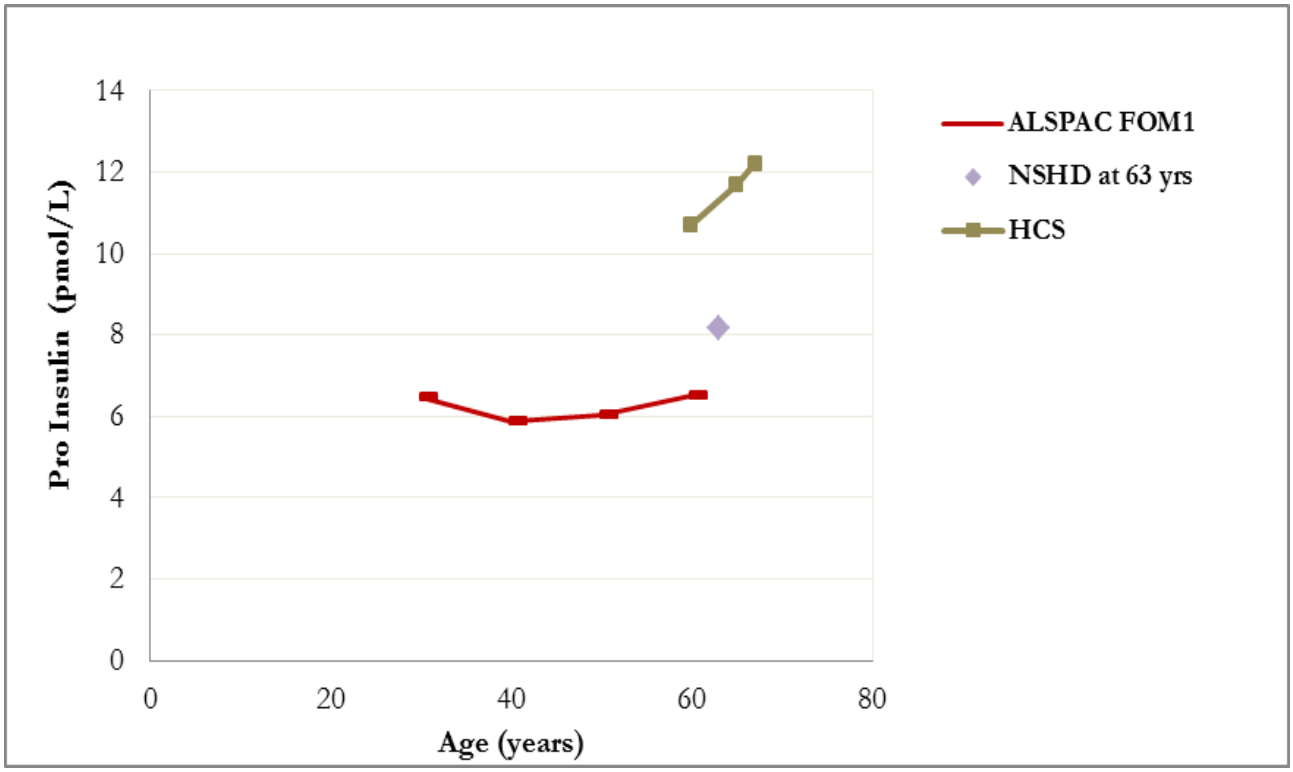
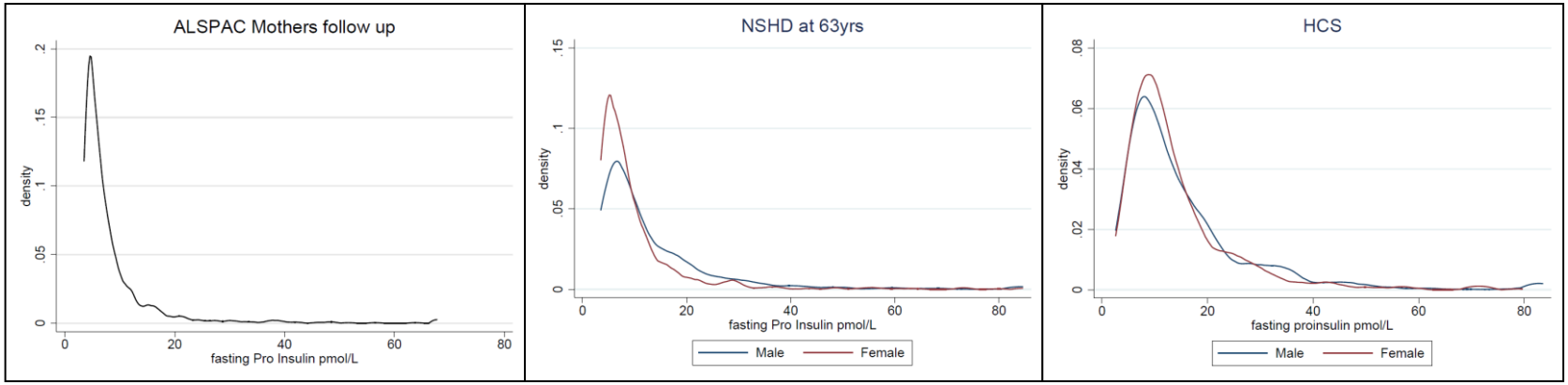


Figure B10. Distributions (Kernel density) of fasting proinsulin (pmol/L) by gender for each CLOSER study that measured proinsulin. Distributions were w insorised at 0.5% and 99.5%.



Glycated Haemoglobin (HbA1c)

Glycated Haemoglobin was measured in four CLOSER studies: ALSPAC, UKHLS, NCDS and NSHD (See **Table 2**).

What is it?

Glycated haemoglobin is an integrated measure of the level of sugar in the blood over the previous eight to 12 weeks before measurement. Technically it is the proportion of haemoglobin proteins that have been bound by glucose[34].

What is its clinical significance?

HbA1c has recently been highlighted as a 'gold standard' indicator of diabetes risk (International Expert Committee, 2009[35]). It can be used both to identify those with diabetes as well as highlight those people who may not be managing their diabetes consistently.

How is it measured?

HbA1c is measured using High Performance Liquid Chromotography in all studies as described in **Table A11**.

UKHLS uses the International Federation of Clinical Chemistry (IFCC) standardisation for HbA1c (mmol/mol). We have harmonised to other studies using the National Glycohemoglobin Standardization Program (NGSP) more common reporting (per cent of haemoglobin that is glycosylated, %). The conversion is based on:

NGSP(%) = 0.09148*IFCC(mmo/mol)+2.152

Are there clinical cut points?

The following cut points are noted:

HbA1c below 42 mmol/mol (6.0%): indicates healthy level, non-diabetic
HbA1c between 42 and 47 mmol/mol (6.0–6.4%): indicates impaired glucose regulation or prediabetes
HbA1c of 48 mmol/mol (6.5%) or over[36,37]: indicates diabetes

What should be considered in analyses?

A number of factors are associated with inaccurate HbA1c measurements[38]; having anaemia, high cholesterol, liver disease, kidney disease, being pregnant or having an uncommon type of Haemoglobin can return inaccurate levels of HbA1c. Diabetic medication (BNF chapter 6.1) will lower HbA1c levels in diabetic individuals.

[34] Nathan D.M. et al. Translating the A1C assay into estimated average glucose values. Diabetes Care 2008; 31: 1473-1478
[35] International Expert Committee (2009). International Expert Committee report on the role of the A1C assay in the diagnosis of diabetes. Diabetes Care 32, 1327–1334.
[36] American Diabetes Association. Executive summary: standards of medical car in diabetes – 2010. Diabetes Care 33 (Suppl 1): S4-S10
[37] World Health Organisation (2011). Use of glycated haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus. Geneva: World Health Organisation
[38] Gallagher E.J., Le Roith D., Bloomgarden Z.Review of haemoglobin A1c in the management of diabetes. J. Diabetes; 2009: 1: 9-17

Table A11. Glycated Haemoglobin, HbA1c (%): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	9 yrs 1 791	Menarini HA 8140 auto-analyser which employs a reverse phase cation exchange HPLC with spectrophotometric detection	4.9 (0.3) 4.9 4.7-5.1	4.9 (0.3) 4.9 4.7-5.1
UKHLS‡	12 162	HPLC cation exchange on a Tosoh G8 analyser	5.6 (0.7) 5.4 5.2-5.7	5.5 (0.7) 5.4 5.2-5.7
SWS	-	-	-	-
NCDS	7 923	Ion exchange high performance liquid chromatography, using the Tosoh A1c 2.2 Glycohemoglobin Analyser HLC-723GHb	5.3 (0.7) 5.2 5.0-5.5	5.2 (0.6) 5.1 4.9-5.3
NSHD	53 yrs 2 582 63 yrs 2 045	Ion exchange high-performance liquid chromatography TOSOH G7 HPLC system.	5.7 (0.6) 5.6 5.3-5.9 5.8 (0.7) 5.7 5.5-6.0	5.6 (0.6) 5.6 5.3-5.9 5.8 (0.6) 5.7 5.5-6.0.
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

‡UKHLS uses the IFCC standardisation. We have harmonised to other studies using the NGSP (%) more common reporting. The conversion is based on: NGSP(%)=0.09148*IFCC+2.152

Figure A11. Crude plot of glycated haemoglobin (%), median, by age for each CLOSER study that measured HbA1c.

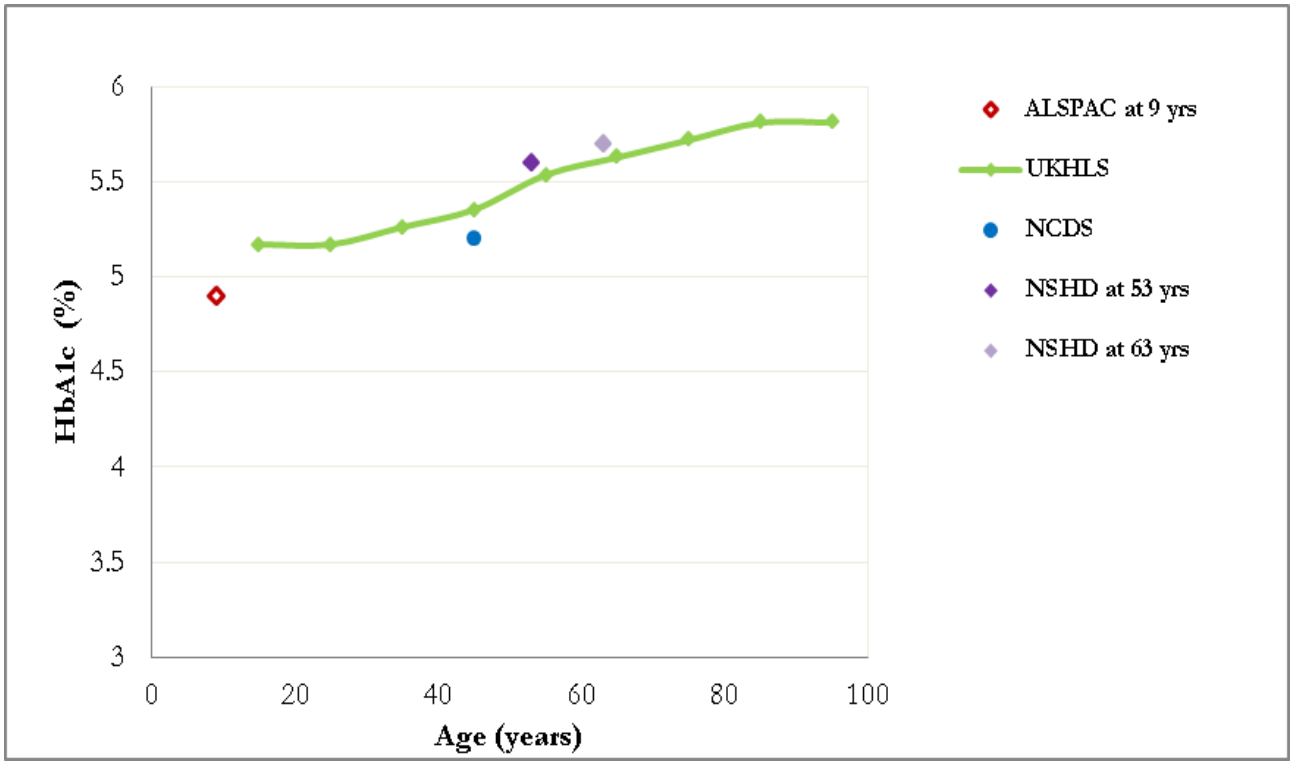
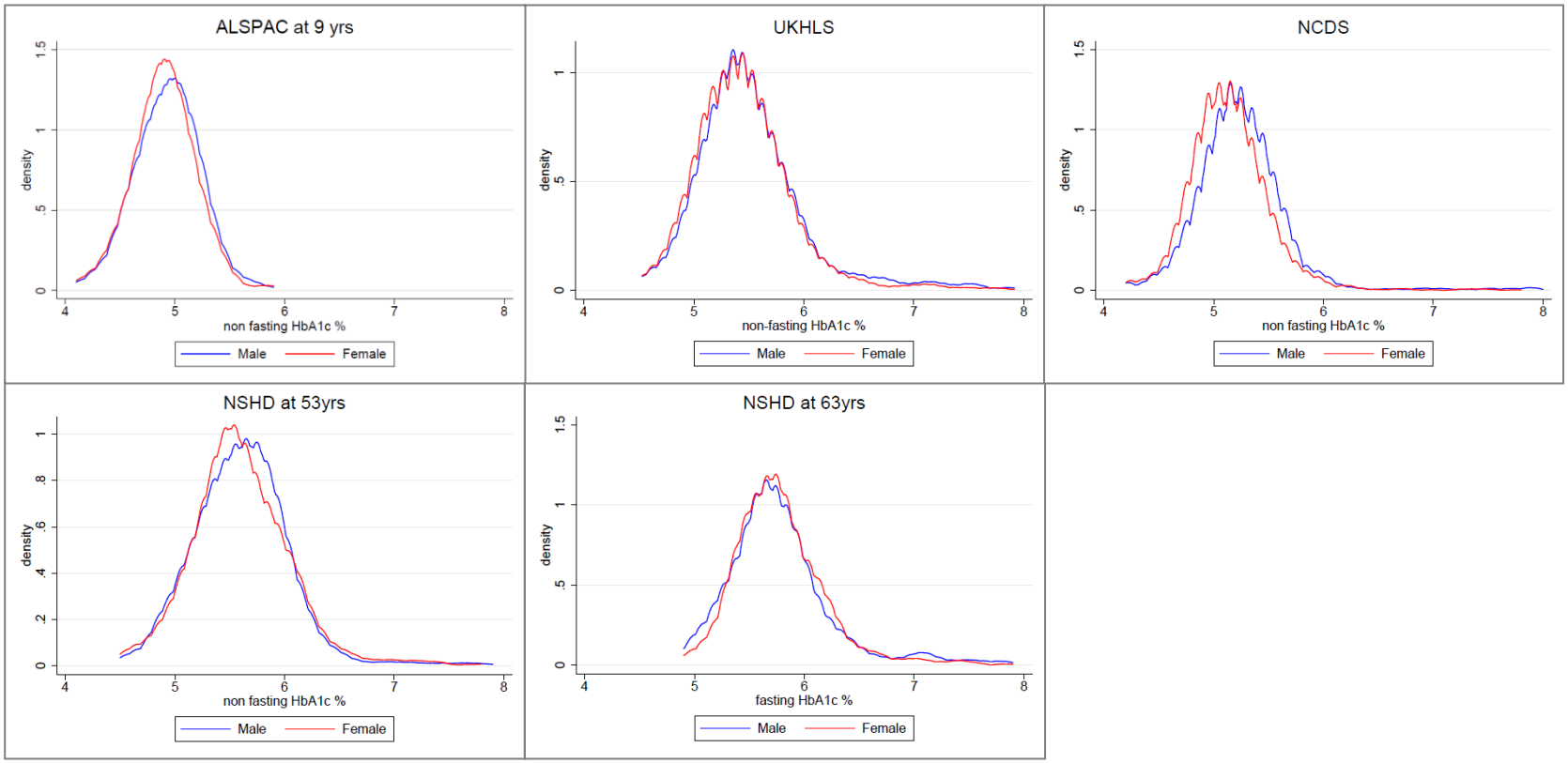


Figure B11. Distributions (Kernel density) of glycated haemoglobin (%) by gender for each CLOSER study that measured HbA1c. Distributions were winsorised at 0.5% and 99.5%.



3.4 Diet & Iron Biomarkers

Ferritin

Haemoglobin (Hb)

Vitamin C

Vitamin D

3.4.1 Association of Diet and Iron markers and the social environment

Anaemia (deficiency of red blood cells or haemoglobin in the blood) and ferritin levels are predictive of mortality, and of both, cardiovascular and non-cardiovascular disease[39],[40]. The association of measures of iron status with social position is not established as there are mixed findings. For example, in pre-menopausal Australian women ferritin levels were raised for those in high school compared to those in higher education with no associations in men or post-menopausal women[41]. In English people aged 50yrs and over, haemoglobin levels show no pattern of association with wealth, but the richest groups appear to be at an advantage with respect to ferritin levels[42].

Low plasma levels of vitamin C appear to be associated with higher risk of cancer incidence and mortality as well as with CVD[43,44]. Further studies have shown that adult and childhood markers of socioeconomic deprivation are related to circulating levels of vitamins C in the British Women's Heart and Health Study and adult social position in the MIDSPAN study[45],[46].

Evidence suggests Vitamin D status is predictive of mortality[47]. Vitamin D levels are lower in older people that had worked in manual vs. non manual occupations [48] and lower values are associated with greater disadvantage in a middle aged Finnish population[49].

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[39] Penninx B.W., et al. Anemia and decline in physical performance among older persons. Am J Med. 2003;115:104–110.
[40] Ellervik C. et al. Total and Cause-Specific Mortality by Moderately and Markedly Increased Ferritin Concentrations: General Population Study and Metaanalysis. Clinical Chemistry 60:11 000–000 (2014)
[41] Ahmed F. et al., Iron status among Australian adults: findings of a population based study in Queensland, Australia. Asia Pac. J. Clin Nutr 2008, 17(1), 40-7
[42] Banks J. et al, Retirement, health and relationships of the older population in England: The 2004 English Longitudinal Study of Ageing (Wave 2).
[43] Khaw K.T., et al. Relation between plasma ascorbic acid and mortality in men and women in EPIC-Norfolk prospective study: a prospective population study. European Prospective Investigation into Cancer and Nutrition. Lancet. 2001;357:657–63.
[44] Moats C., Rimm E.B. Vitamin intake and risk of coronary disease: observation versus intervention. Curr Atheroscler Rep. 2007;9:508–14.
[45]Lawlor D.A. et al. Vitamin C is not associated with coronary heart disease risk once life course socioeconomic position is taken into account: prospective findings from the British Women's Heart and Health Study. Heart. 2005 Aug; 91(8):1086-7.
[46] Talwar D. et al. Which circulating antioxidant vitamins are confounded by socioeconomic deprivation? The MIDSPAN family study. PLoS One. 2010 Jun 25;5(6):e11312.
[47]Schöttker B. et al. Vitamin D and mortality: meta-analysis of individual participant data from a large consortium of cohort studies from Europe and the United States. BMJ 2014;348:g3656
[48] Hirani V., Primatesta P. Vitamin D concentrations among people aged 65 years and over living in private households and institutions in England: population survey. Age Ageing. 2005 Sep;34(5):485-91.
[49] Palaniswamy S. et al, Potential determinants of vitamin D in Finnish adults: a cross-sectional study from the Northern Finland birth cohort 1966. BMJ Open. 2017 Mar 6;7(3):e013161.

Ferritin

Ferritin has been measured in four CLOSER studies: ALSPAC, UKHLS, SWS and NSHD (see **Table 2**).

What is it?

Ferritin is an iron storage protein and a marker of iron stores.

What is its clinical significance?

Levels of ferritin reflect the size of the body iron stores and therefore it is indicative of anaemia. A low ferritin level is predictive of uncomplicated iron deficiency anaemia. However, a high plasma ferritin level can detect excess body iron, which is also problematic for overall health.

How is it measured?

Ferritin is measured as described in **Table A12**.

Are there clinical cut points?

Clinical cut points are gender specific; both high and low levels of ferritin are associated with adverse outcomes. The following cut points are suggested[50].

Clinical cut-points for ferritin in men and women
≤ 20 ng/mL indicates depletion of ferritin, while ≤ 12 ng/mL indicates complete absence of stored iron
>300 ng/mL may indicate iron overload in men and postmenopausal women and >200 may indicate iron overload in premenopausal women

What should be considered in analyses?

Ferritin levels are influenced by anti-inflammatory medication[51] (BNF chapter 2.9, BNF chapter 10.1). Low ferritin levels often mean an iron deficiency is present. This can be caused by long-term (chronic) blood loss from heavy menstrual bleeding, pregnancy, not enough iron in the diet, or bleeding inside the intestinal tract (from ulcers, colon polyps, colon cancer, haemorrhoids or other conditions). Ferritin levels can be high due to inflammatory diseases and liver damage.

[50] World Health Organisation. Serum ferritin concentrations for the assessment of iron status and iron deficiency in populations. Vitamin and Mineral Nutrition Information System. Geneva, World Health Organization, 2011

[51] Fleming D.J. et al. Aspirin intake and the use of serum ferritin as a measure of iron status Am J Clin Nutr 2001; 74:2 219-226

Table A12. Ferritin (ng/mL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 99.5%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	8 mths	754	DELFIA time resolved fluoroimmunoassay system	41.1 (26.1) 35.2 25.3-49.3	48.7 (28.2) 41.3 29.1-60.5
	12 mths	752		34.6 (17.7) 30.5 23.2-45.9	38.4 (19.2) 34.5 25.1-45.9
	18 mths	733		30.5 (17.9) 25.2 18.7-37.3	32.4 (17.8) 28.0 30.4-39.8
UKHLS	12 894		Electrochemiluminiscent immune assay on the ROCHE Modular E170 analyser	184.9 (142) 148.0 91.0-232.0	93.9 (84.3) 70.0 38.0-120.0
SWS	LP‡	501	Sandwich ELSA methods on a commercial ELISA kit (AssayMax Human Ferritin ELISA, AssayPro)	-	57.8 (36.6) 48.3 32.4-75.4
NCDS	-		-	-	-
NSHD	53 yrs	2 497	Specific immunoassays on an Abbott Imx analyzer	144.4 (116.2) 118.0 75.5-179.1	70.0 (75.4) 54.0 (33.0-85.0)
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**
‡ LP: Approximate gestation age is 34 weeks.

Figure A12. Crude plot of ferritin (ng/mL), median by age for each CLOSER study that measured ferritin.

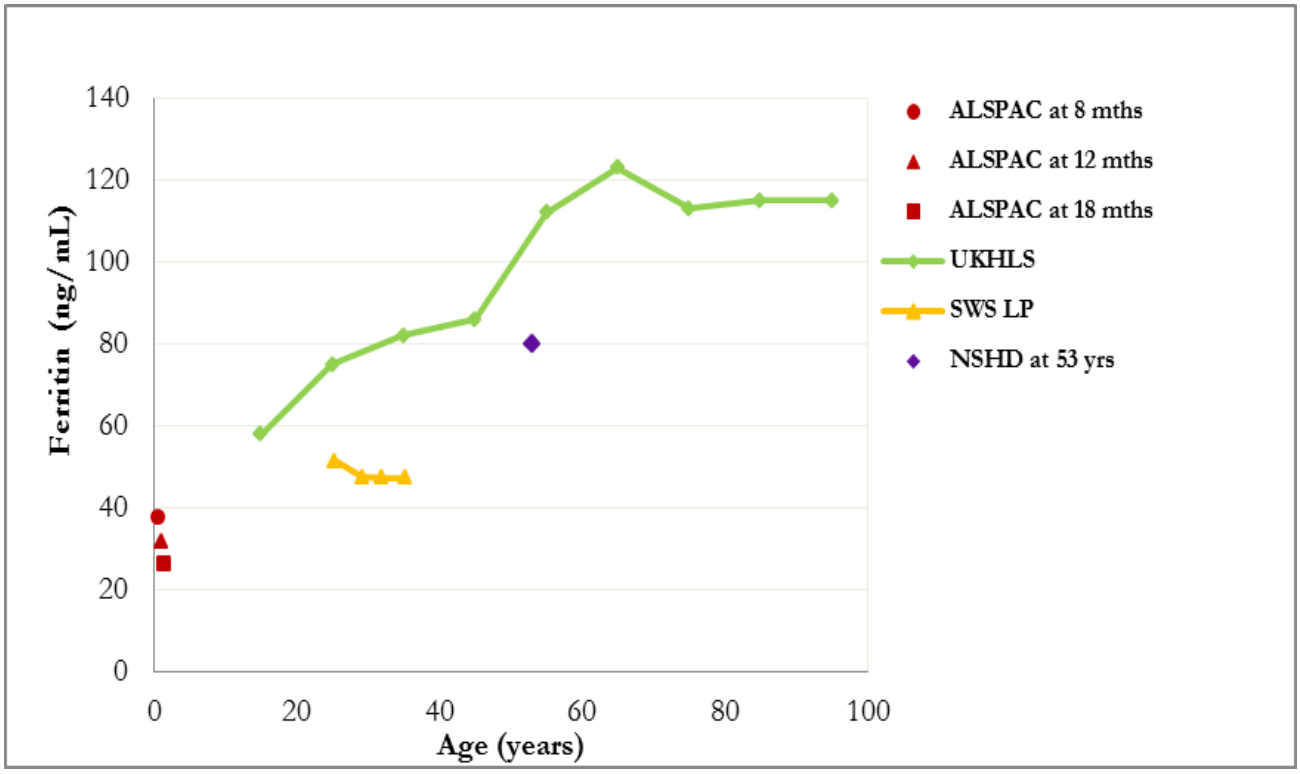
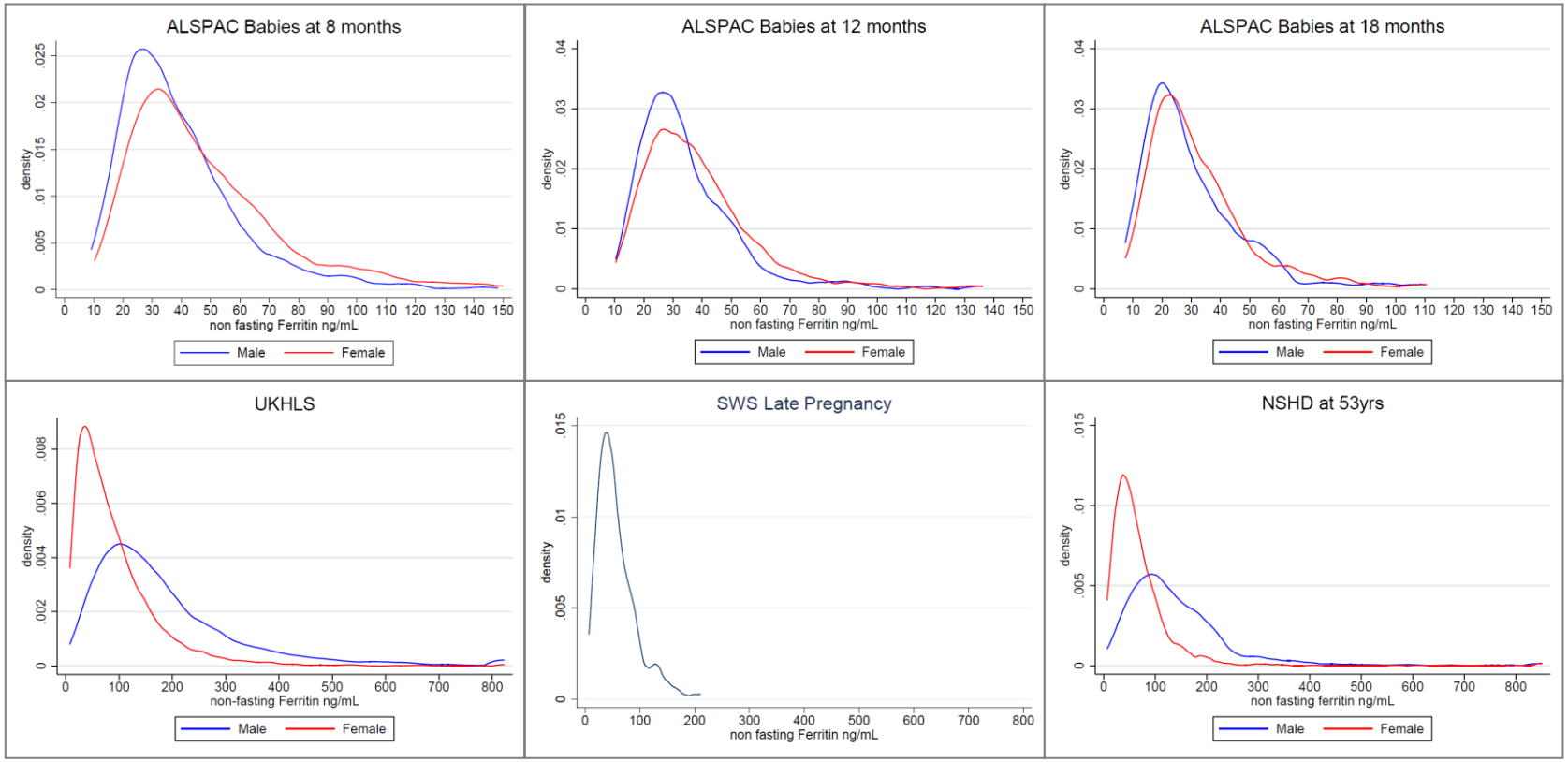


Figure B12. Distributions (Kernel density) of ferritin (ng/mL) by gender for each CLOSER study that measured ferritin. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



Haemoglobin (Hb)

Haemoglobin has been measured in four CLOSER studies: ALSPAC, UKHLS, SWS and NSHD (see **Table 2**).

What is it?

Haemoglobin is the iron-containing molecule responsible for carrying oxygen from the respiratory organs to the rest of the body; low levels are usually indicative of anaemia.

What is the clinical significance of haemoglobin?

Low levels of haemoglobin are suggestive of anaemia, a lack of iron in the blood, which is prevalent in the elderly[52]. It is associated with longer hospitalization, greater risk of mortality and CVD[53].

How is it measured?

Haemoglobin is measured as described in **Table A13.** Ideally, participants should be reasonably hydrated when having a haemoglobin test or the result may be inaccurately high.

Are there clinical cut points?

Anaemia status (no/yes) is defined based on WHO guidelines (<130 g/L for men and <120 g/L for women)[54]. Normal levels of haemoglobin are influenced by age, sex and ethnic origin.

What should be considered in analyses?

Haemoglobin levels are influenced by a number of factors, such as pregnancy and high altitude but the latter is not applicable to our population.

Above-normal haemoglobin levels may be the result of dehydration, excess production of red blood cells in the bone marrow, severe lung disease, or several other conditions.

Below-normal haemoglobin levels may be the result of iron deficiency,
eficiencies, kidney disease, inflammatory disorders such as rheumatoid arthritis or infections, haemolysis (accelerated loss of red blood cells through destruction), inherited haemoglobin defects such as thalassaemia or sickle cell anaemia, cirrhosis of the liver, bone marrow failure, cancers that affect the bone marrow, infection.
Haemoglobin concentration decreases slightly during normal pregnancy. Haemoglobin levels peak around 8 a.m. and are lowest around 8 p.m. each day. Heavy smokers have higher haemoglobin levels than non-smokers. Haemoglobin levels are slightly lower in older men and women and in children.

[52] Nilsson-Ehle H. et al. Blood haemoglobin declines in the elderly: implications for reference intervals from age 70 to 88. Eur. J. Haematol., 65 (2000), 297–305

[53] Culleton B.F. et al. Impact of anemia on hospitalization and mortality in older adults Blood, 107 (2006), 3841–3846

[54] WHO (World Health Organization) Nutritional Anaemias: Report of a WHO Scientific Group. World Health Organization, Geneva (1968)

Table A13. Haemoglobin, Hb (g/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	8 mths	1 074	Assayed using the HEMOCUE B-Hb photometer	116.7 (11.4) 117.0 109-129	117.4 (10.8) 118.0 111-125
	12 mths	815		117.3 (10.2) 118.0 111-124	117.8 (10.3) 117.5 112-125
	18 mths	920		116.2 (9.1) 117.0 110-122	117.3 (8.8) 118.0 112-122
	31 mths	584		115.3 (8.0) 116.0 110-121	117.2 (8.2) 117.0 113-122
	43 mths	718		118.0 (10.6) 118.0 113-125	119.5 (9.8) 120.0 114-125
	61 mths	622		118.0 (8.3) 119.0 114-124	120.5 (9.7) 122.0 116-127
	7 yrs	5 844		124.6 (7.6) 125.0 120-129	124.3 (7.6) 124.0 119-129
	9 yrs	5 185		127.5 (9.6) 128.0 122-134	126.3 (9.7) 127.0 121-133
	11+ yrs	4 715		127.8 (9.8) 128.0 122-134	127.0 (9.7) 128.0 121-133
	13.5 yrs	3 247		133.5 (10.4) 133.0 127-140	126.7 (9.3) 127.0121-133
	15.5 yrs	3 523		145.0 (11.0) 146.0 138-152	130.8 (10) 131.0 125-138
	17.5 yrs	3 318		149.7 (9.9) 150.0 143-156	130.9 (9.9) 131.0 125-137
	FOF1, Fathers	1 965		148.9 (9.9) 149.0 143-155	-
	FOM1, Mothe33rs	4 186		-	129.7 (16.4) 131.0 125-138
UKHLS	12 156		Spectrophotometric assay on a Sysmex XE-2100 analyser	145.4 (11.7) 146.0 139-153	130.2 (10.8) 130.0 124-137
SWS	Init	7 750		-	129.6 (8.9) 130.0 124-135
	EP†	1 980		-	126.7 (8.6) 127.0 121-132
	LP‡	2 309		-	115.6 (9.4) 115.0 109-122
NCDS	-		-	-	-
NSHD	63 yrs	2 062	Standard spectrophotometric analysis on a Coulter Electronics STKS analyzer	149 (11.0) 150.0 142-157	136 (9.0) 136.0 130-142
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**
†EP: Approximate gestation age is 11 weeks
‡LP: Approximate gestation age is 34 weeks.

Figure A13. Crude plot of haemoglobin (g/L), median, by age for each CLOSER study that measured Hb.

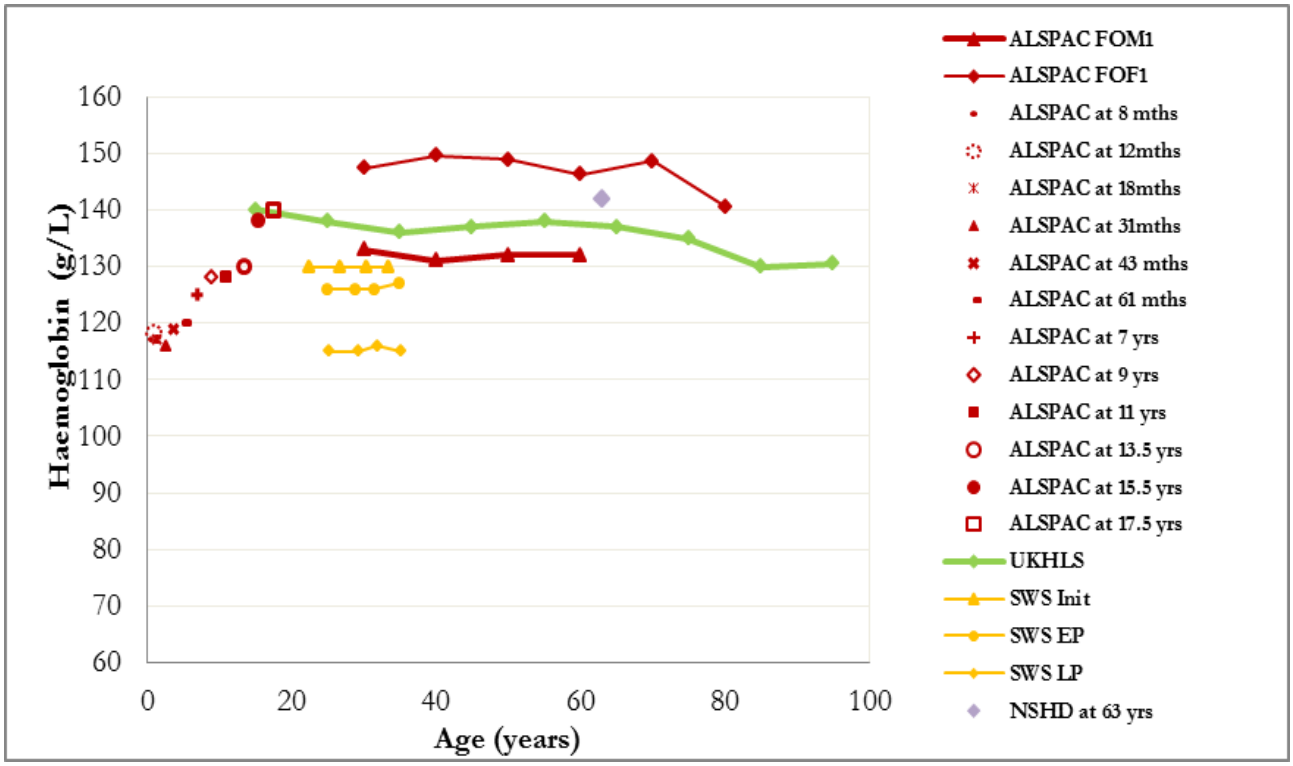
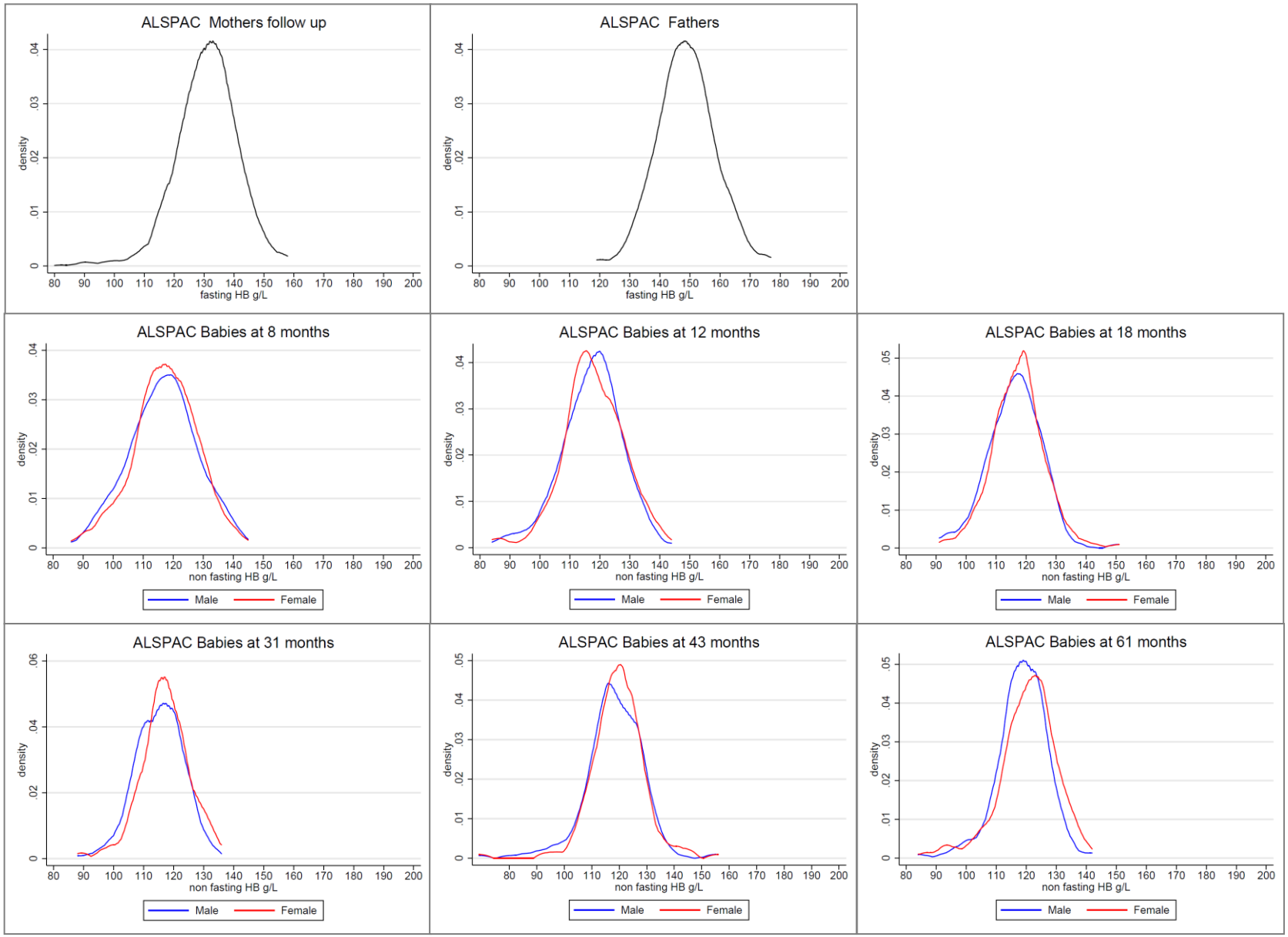
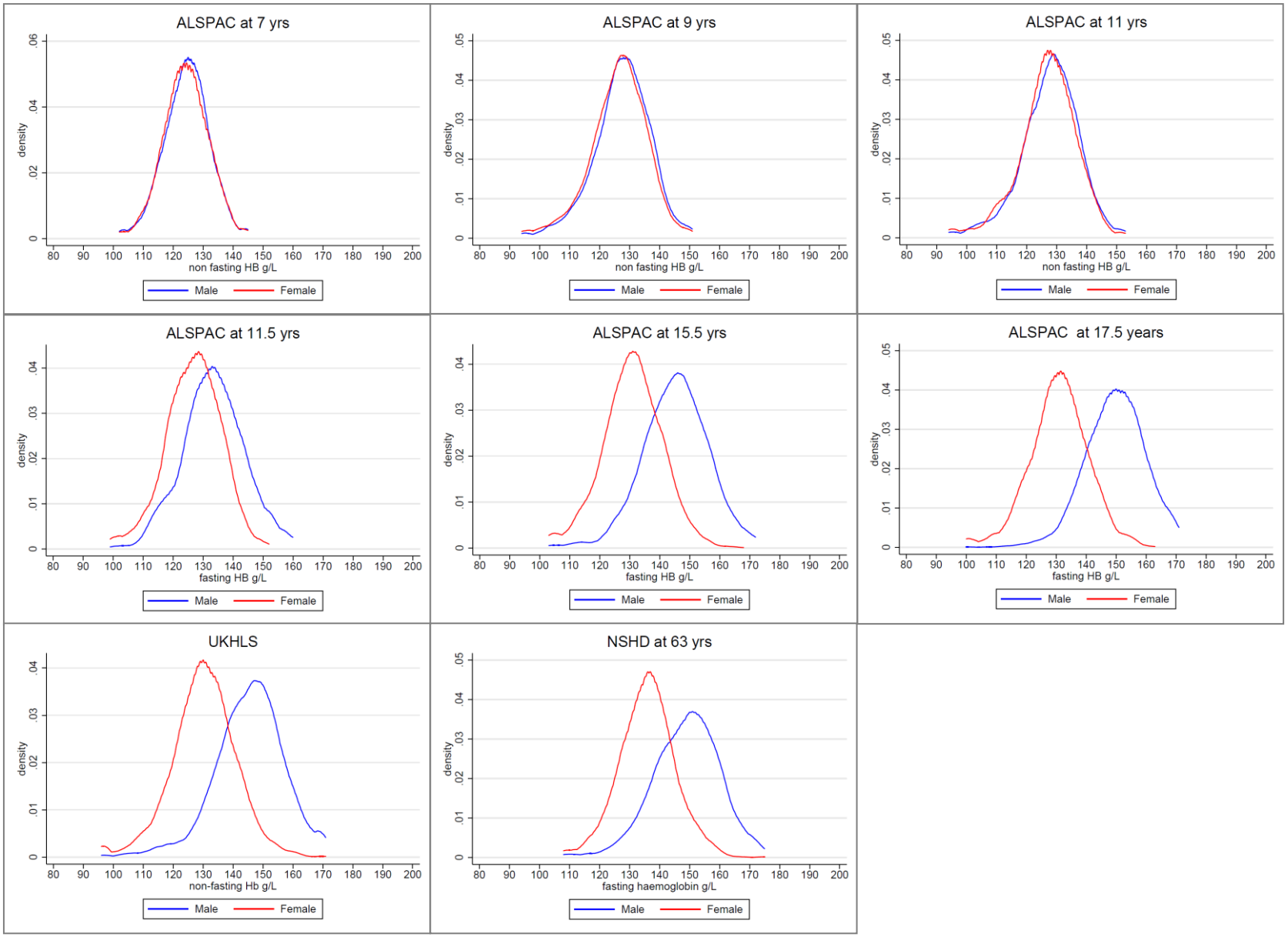
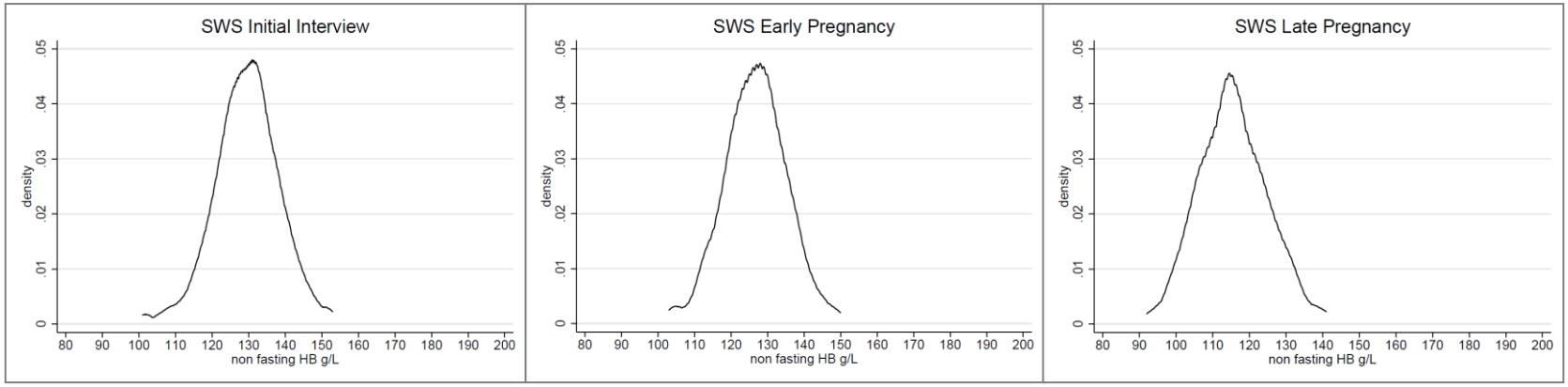


Figure B13. Distributions (Kernel density) of haemoglobin (g/L) by gender for each CLOSER study that measured Hb. Distributions were winsorised at 0.5% and 99.5%.




ALSPAC at 7 yrs
density
non fasting HB g/L
Male
Female
ALSPAC at 9 yrs
density
non fasting HB g/L
Male
Female
ALSPAC at 11 yrs
density
non fasting HB g/L
Male
Female
ALSPAC at 11.5 yrs
density
fasting HB g/L
Male
Female
ALSPAC at 15.5 yrs
density
fasting HB g/L
Male
Female
ALSPAC at 17.5 years
density
fasting HB g/L
Male
Female
UKHLS
density
non-fasting Hb g/L
Male
Female
NSHD at 63 yrs
density
fasting haemoglobin g/L
Male
Female


SWS Initial Interview
SWS Early Pregnancy
SWS Late Pregnancy
density
0
.01
.02
.03
.04
.05
80 90 100 110 120 130 140 150 160 170 180 190 200
non fasting HB g/L

Vitamin C

Vitamin C (Ascorbic Acid) has been measured in two CLOSER studies: SWS and NSHD (see **Table 2**).

What is it?

Vitamin C, also known as ascorbic acid, is a water-soluble nutrient found in some foods; the human body does not produce vitamin C. vitamin C acts as an antioxidant, helping to protect cells from the damage caused by free radicals.

How is it measured?

Vitamin C is measured using in-house methods as described in **Table A14.** Detailed protocols are available from the studies that have measured vitamin C.

What is the clinical significance of Vitamin C?

Vitamin C is required for the proper development and function of many parts of the body. It also plays an important role in maintaining proper immune function.

Are there clinical cut points?

There are no clinical cut points for serum levels of vitamin C. There are no standardized methods for the measurement of vitamin C.

What should be considered in analyses?

Low levels of vitamin C are seen in people dependent on drugs and/or alcohol, people on low income or the elderly who may not have a healthy, balanced diet; those presenting with medical conditions that affect the body's ability to digest and absorb food, such as Crohn's disease and ulcerative colitis will have low vitamin C levels. Smoking affects the absorption of vitamin C from foods and also vitamin C is used up in the body more quickly in those who smoke. Pregnant and breast-feeding women show lower levels of vitamin C. Low vitamin C concentrations can also occur in patients with end-stage renal disease or chronic haemodialysis.

Table A14. Vitamin C (μmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	-		-	-	-
UKHLS	-		-	-	-
SWS	LP†	1 265	In-house methodology	-	39.1 (1.1) 39.3 22.4-53.8
NCDS	-		-	-	-
NSHD	63 yrs	1 851	In-house methodology	50.9 (18.0) 52.4 39.8-63	61.4 (18.7) 63.0 51.9-73.2
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**
‡LP: Approximate gestation age is 34 weeks.

Figure A14. Plot of Vitamin C (µmol/L), median, by age for each CLOSER study that measured Vitamin C.

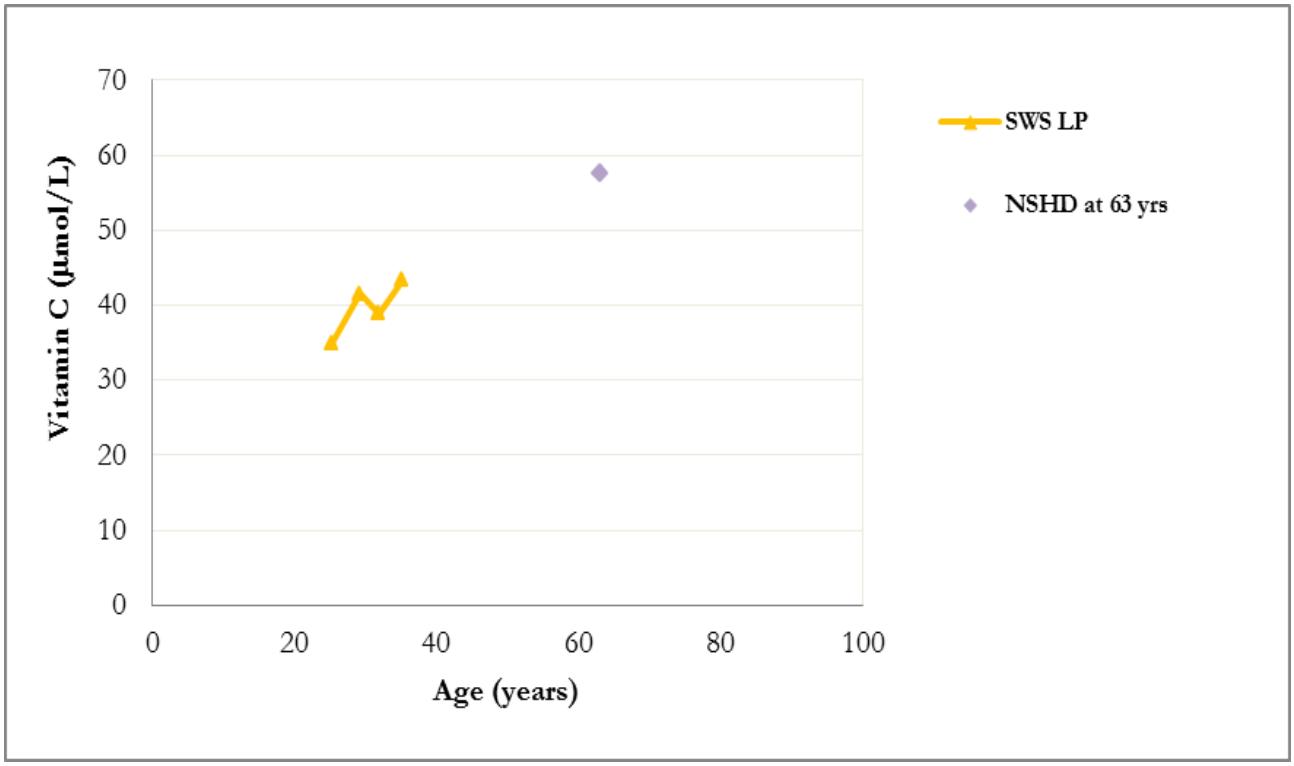
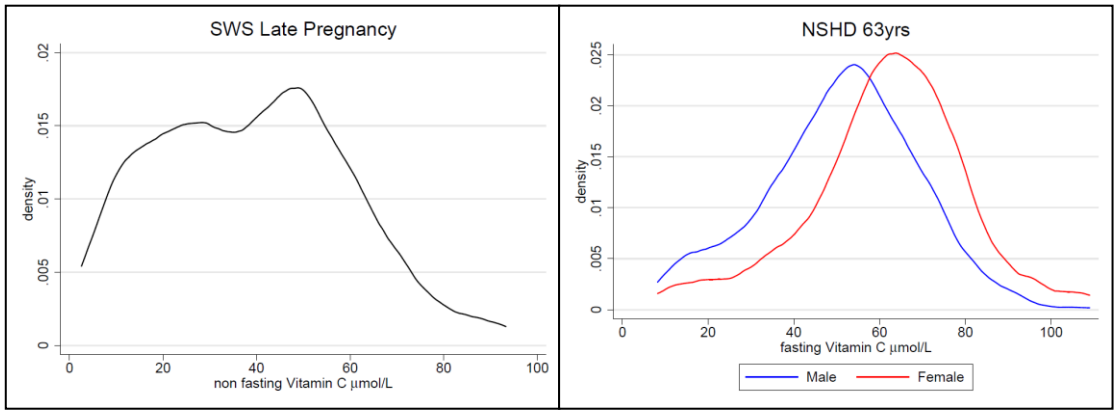


Figure B14. Distributions (Kernel density) of Vitamin C (µmol/L) by gender for each CLOSER study that measured Vitamin C. Distributions were winsorised at 0.5% and 99.5%.



Vitamin D

Total vitamin D has been measured in three CLOSER studies: ALSPAC, SWS and NSHD (see **Table 2**).

In addition, components of total vitamin D, vitamins D_2 and D_3, have been measured in ALSPAC; while NCDS is the only study measuring vitamin D from saliva samples (not reported here).

What is it?

Vitamin D is an organic compound responsible for increasing intestinal absorption of calcium, iron, magnesium, phosphate, and zinc. Vitamin D mainly comes from the skin when it is exposed to sunlight.

How is it measured?

Total vitamin D in serum is measured using high performance liquid chromatography or radioimmunoassay methods as described in **Table A15.**
A common technique to measure vitamin D in serum is by separating and quantifying vitamin D_3 (cholecalciferol) and vitamin D_2 (ergocalciferol), the vitamers of most interest in the assessment of vitamin D status in humans[55].
Vitamin D_3 is present in serum at a higher concentration than vitamin D_2 (which may not be present at all). Laboratory reports for NSHD indicate non-detectable serum vitamin D_2 levels for 99% of their participants (the level of detection, LOD, was 18.2 nmol/L for vitamin D_2). Thus, total vitamin D for NSHD as presented here, corresponds to vitamin D_3 reported by the laboratory.
ALSPAC measured total vitamin D levels, in addition to vitamins D_2 and D_3, but we only report total vitamin D.

What is the clinical significance of Vitamin D?

Vitamin D helps to regulate the amount of calcium and phosphate in the body. These nutrients are needed to keep bones, teeth and muscles healthy. A lack of vitamin D can lead to bone deformities such as "rickets" in children, and bone pain and tenderness as a result of a condition called osteomalacia in adults.

Are there clinical cut points?

The level of serum vitamin D for a healthy individual is recommended at an optimum of 75 nmol/L (30 ng/mL)[54].

What should be considered in analyses?

A number of factors influence vitamin D levels; these factors include variation in sun exposure due to latitude of residence, season, time of day, atmospheric components, clothing, sunscreen use and skin colour, as well as age, obesity, pregnancy, vitamin supplementation and the incidence of several chronic illnesses[56,57]. Serum vitamin D is relatively stable and not directly influenced by diet (e.g., calcium intake) and life style (e.g., mobility)[58].

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[55] Pearce S.H.S. and Cheetham T.D. Diagnosis and management of vitamin D deficiency. Brit Med J 2010;340:142-147
[56] Tsiaras W.G. and Weinstock M.A. Factors influencing vitamin D status. Acta Derm Venereol, 91(2), 2011, 115-24
[57] Schramm S. et al. Impact of season and different vitamin D thresholds on prevalence of vitamin D deficiency in epidemiological cohorts-a note of caution. Endocrine. 2017 Jun; 56(3):658-666
[58] Lips P. Relative Value of 25(OH)D and $1,25(OH)_2D$ Measurements. Journal of Bone and Mineral Research. 2007. 22(11):1668-71

Table A15. Vitamin D (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy†	7 952	High performance liquid chromatography tandem mass spectrometry	-	66.8 (32.0) 61.3 49.2-84.4
	7 yrs	1 056		79.3 (32.1) 74.6 55.4-97.8	78.5 (27.8) 75.9 59.4-95.3
	9 yrs	4 442		61.9 (19.3) 60.7 48.2-73.6	60.1 (18.2) 58.9 47.2-71.1
	11^{+} yrs	1 008		62.2 (18.0) 59.7 49.2-71.9	55.9 (17.4) 54.2 44.2-67.1
UKHLS	-		-	-	-
SWS	LP‡	2 332	DiaSorin radioimmunoassay (RIA)	-	64.3 (0.8) 58.1 40.2-83.7
NCDS	-		-	-	-
NSHD	63 yrs	1 220	High performance liquid chromatography tandem mass spectrometry	38.9 (18.6) 37.0 25.0-49.0	38.8 (18.8) 35.0 26.0-50.0
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

†Mean (SD) gestation of blood sample is 25 (11) weeks.

‡LP: Approximate gestation age is 34 weeks.

Figure A15. Plot of Vitamin D (nmol/L), median, by age for each CLOSER study that measured Vitamin D.

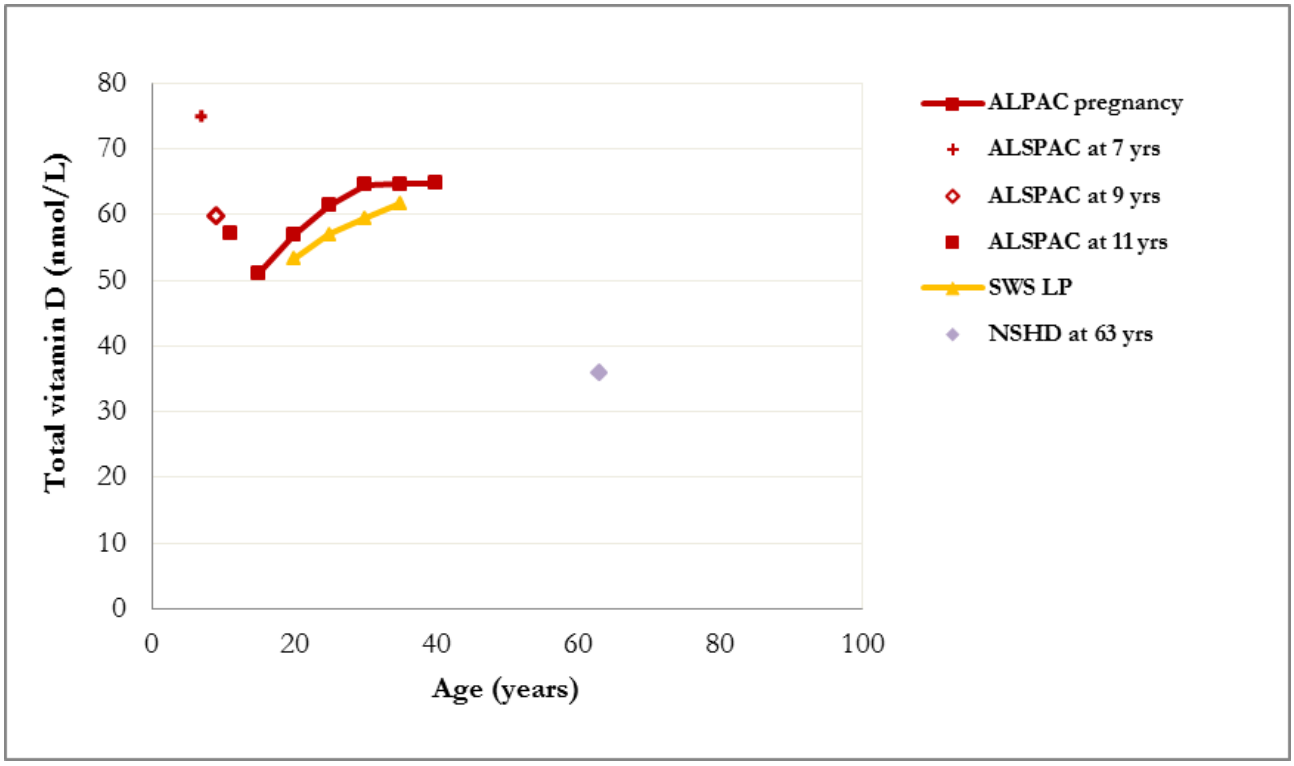
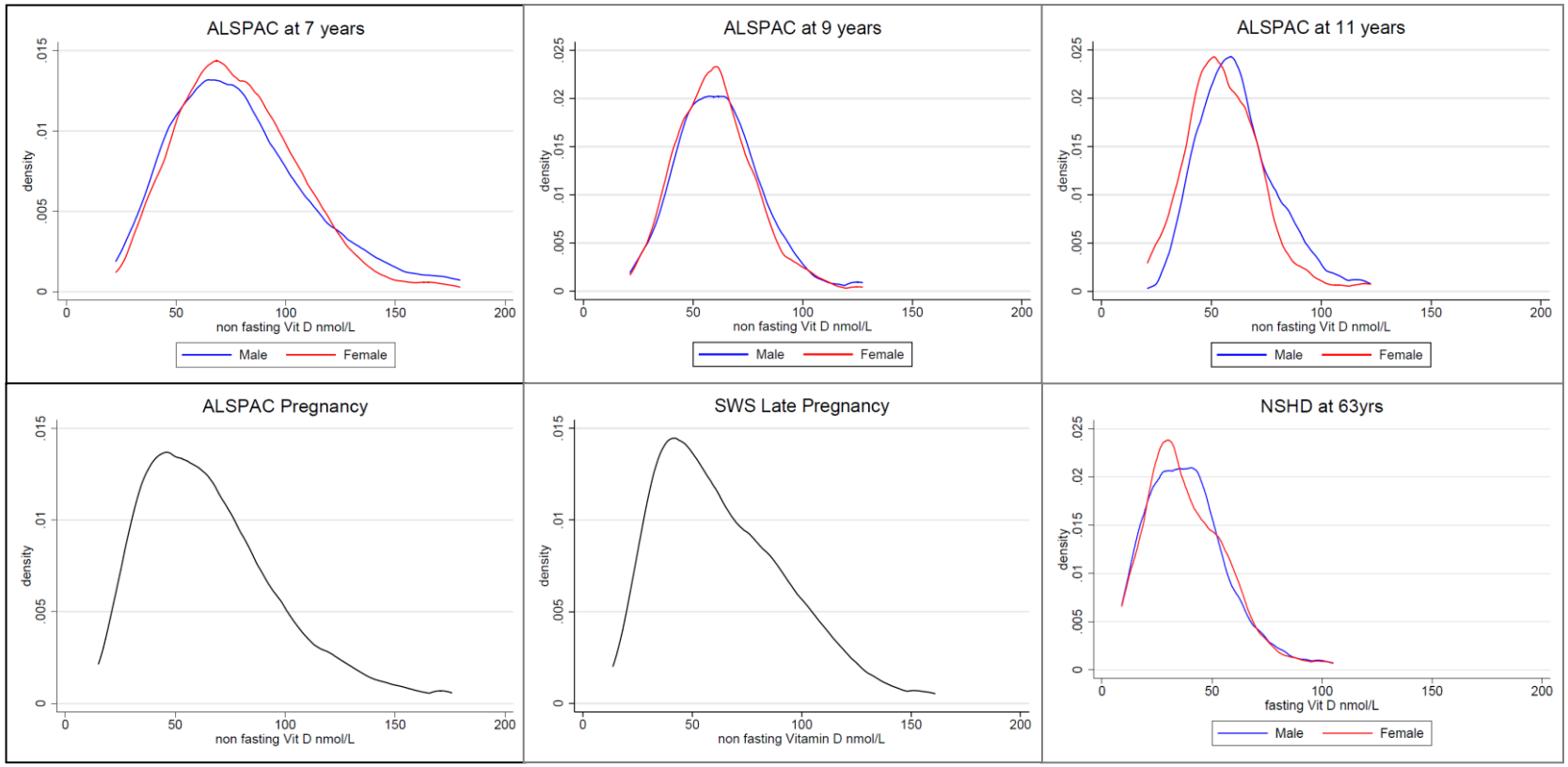


Figure B15. Distributions (Kernel density) of Vitamin D (nmol/L) by gender for each CLOSER study that measured Vitamin D. Distributions were winsorised at 0.5% and 99.5%.



3.5 Inflammatory Biomarkers

C-Reactive Protein (CRP)

Fibrinogen

Immunoglobulin E (IgE)

Interleukin-6 (IL-6)

Red Cell Folate

Tissue Plasminogen Activator (TPA)

Von Willebrand Factor (VWF)

Platelet count

Red Blood Cells (RBC) count

White Blood Cells (WBC) count

3.5.1 Association of Inflammatory markers and the social environment

Inflammatory markers have emerged as markers that may mediate the association of social position and health. Inflammatory markers have been associated with mortality[59],[60]. Some of the inflammatory markers included here have additional functions such as haemostasis (fibrinogen) and endothelial function (von Willebrand factor, VWF). Tissue plasminogen activator is not an inflammatory marker but a marker of haemostasis.

C-reactive protein[61,62,63,64], fibrinogen[48,49,65] interleukin-6[50] and VWF[66] are associated with social position such that disadvantaged groups have increased levels of inflammatory markers. Tissue plasminogen activator is not socially patterned independently of health behaviours[49]. Red cell folate has not been examined in this context.

[59] Wennberg P. et al. Haemostatic and inflammatory markers are independently associated with myocardial infarction in men and women. Thromb Res. 2012 Jan;129(1):68-73.
[60] Kaptoge S. et al. C-reactive protein, fibrinogen, and cardiovascular disease prediction. Emerging Risk Factors Collaboration. N Engl J Med. 2012 Oct 4;367(14):1310-20
[61] Hughes A. et al. Elevated inflammatory biomarkers during unemployment: modification by age and country in the UK. J Epidemiol Community Health. 2015 Jul;69(7):673-9.
[62] Tabassum F. et al. Effects of socioeconomic position on inflammatory and hemostatic markers: a life-course analysis in the 1958 British birth cohort. Am J Epidemiol. 2008 Jun 1;167(11):1332-41.
[63] Gimeno D. Adult socioeconomic position, C-reactive protein and interleukin-6 in the Whitehall II prospective study. Eur J Epidemiol. 2007;22(10):675-83.
[64] Stringhini S. et al. Lifecourse socioeconomic status and type 2 diabetes: the role of chronic inflammation in the English Longitudinal Study of Ageing. Sci Rep. 2016 Apr 22;6:24780.
[65] Brunner E.J. et al. Social inequality in coronary risk: central obesity and the metabolic syndrome. Evidence from the Whitehall II study. Diabetologia. 1997 Nov;40(11):1341-9.
[66] Kumari M., Marmot M., Brunner E. Social determinants of von willebrand factor: the Whitehall II study. Arterioscler Thromb Vasc Biol. 2000 Jul;20(7):1842-7

C-Reactive Protein (CRP)

C-reactive protein is measured in five CLOSER studies: ALSPAC, UKHLS, NCDS, NSHD and HCS (see **Table 2**).

What is it?

CRP is a protein produced by the liver in response to inflammation. It is part of the body's defence mechanism against harmful stimulus.

What is its clinical significance?

CRP is a marker of inflammatory load that has been shown to predict adverse cardiovascular outcomes and mortality[67].

Systemic inflammation is defined at CRP > 3 mg/L, based on the clinical guidelines of the joint scientific statement from the Centers for Disease Control and Prevention (CDC) and American Heart Association (AHA) that CRP levels above 3 mg/L be used to indicate high risk of cardiovascular diseases[68].

How is it measured?

CRP is measured as described in **Table A16**.

Are there clinical cutpoints?

Values of >3 mg/L are considered a risk factor for cardiovascular disease.[18]

What should be considered in analyses?

Values >10mg/L are considered to reflect recent infection. It is recommended that these data should be removed prior to analyses unless recent infection is a focus of analysis, although some healthy adults do present with high levels of CRP[69].

There is no single mathematical transformation that can transform the highly skewed distribution of CRP to a normal distribution; researchers should carefully consider the most suitable methodology for statistical analysis and interpretation of results.

CRP is influenced by medication: anti-inflammatory medications (BNF Chapter 10.1), statins (BNF Chapter 2.12) and hormone replacement therapy (BNF Chapter 6.4.1).

There may also be a diurnal variation in CRP levels and analysts should check this in their analyses[70] .

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[67] Libby P., Ridker P.M., Hansson G.K. Progress and challenges in translating the biology of atherosclerosis. Nature, 473 (2011), 317–325

[68] Pearson T.A. et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: A statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation, 107 (2003), 499–511

[69] Shine B, de Beer F.C. and Pepys M.B. Solid phase radioimmnoassays for human C-reactive protein. Clinica Chimica Acta, 117 (1981) 13-23.

[70] Rudnicka A.R. et al. Diurnal, Seasonal, and Blood-Processing Patterns in Levels of Circulating Fibrinogen, Fibrin D-Dimer, C-Reactive Protein, Tissue Plasminogen Activator, and von Willebrand Factor in a 45-Year-Old Population. Circulation, 2007;115:996-1003

Table A16. C-Reactive Protein, CRP (mg/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%. Values >10mg/L are not included in calculations.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	9 yrs	5 082	Automated particle-enhanced immunoturbidimetric assay	0.5 (0.9) 0.2 0.1-0.4	0.7 (1.1) 0.3 0.1-0.7
	15.5 yrs	3 488		0.8 (1.3) 0.4 0.2-0.9	0.9 (1.3) 0.4 0.2-0.9
	17.5 yrs	3 287		0.9 (1.2) 0.4 0.3-0.9	1.3 (1.5) 0.7 0.3-1.6
	FOF1, Fathers	1 905		1.7 (1.6) 1.1 0.6-2.1	-
	FOM1, Mothers	4 159		-	1.7 (1.8) 1.0 0.5-2.1
UKHLS	12 530		N Latex CRP mono Immuno assay on the Behring Nephelometer II analyser	1.9 (1.9) 1.2 0.6-2.5	2.2 (2.1) 1.5 0.7-3.0
SWS	-		-	-	-
NCDS	7 692		High-sensitivity nephelometric analysis of latex particles coated with CRP monoclonal antibodies (BN ProSpec protein analyzer)	1.5 (1.6) 0.9 0.5-1.9	1.8 (2.0) 1.0 0.4-2.3
NSHD	63 yrs	2 065	Assayed by nephelometry (Dade Behring)	2.5 (1.8) 1.9 0.8-3.2	2.7 (2.0) 2.0 1.3-3.4
HCS	382		Multiplex technology (HAS)	1.5 (1.6) 0.8 0.4-2.0	1.9 (2.0) 1.1 0.5-2.4

***Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A16. Crude plot of C-reactive protein (mg/L), median, by age for each CLOSER study that measured CRP.

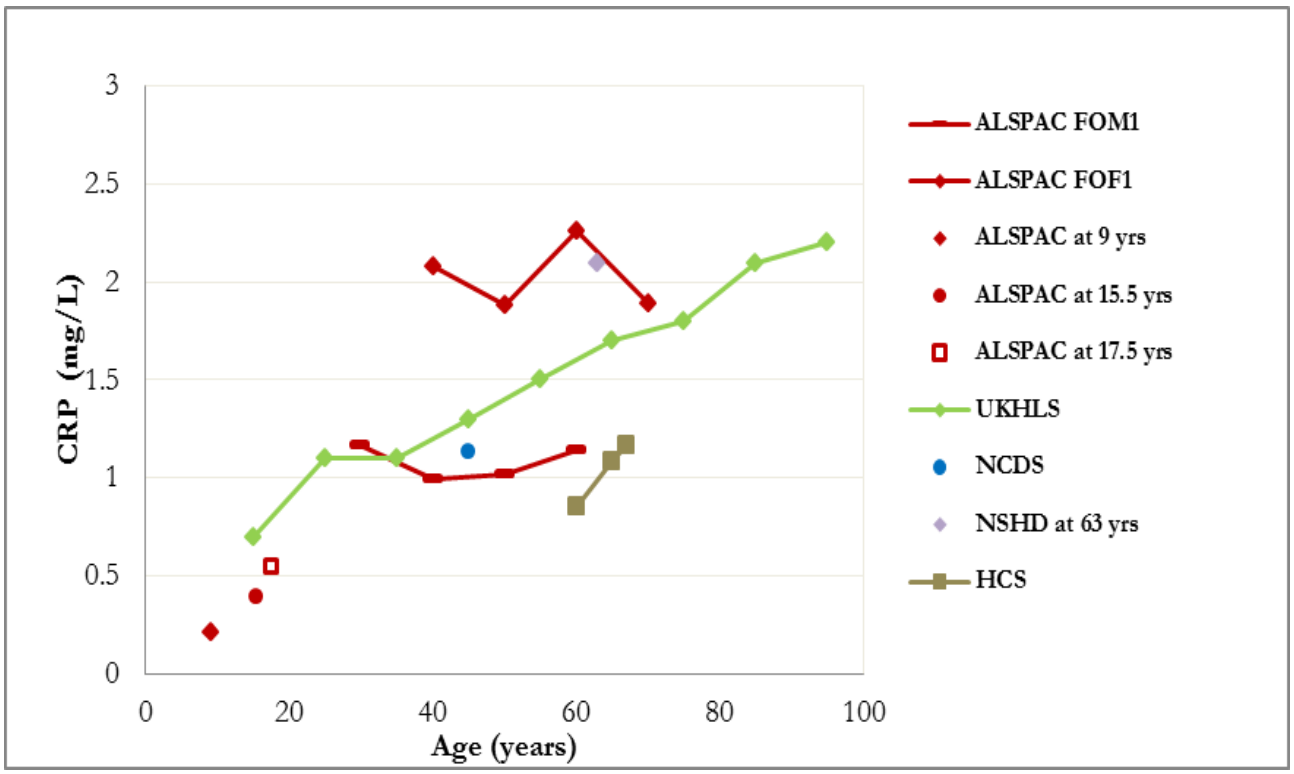
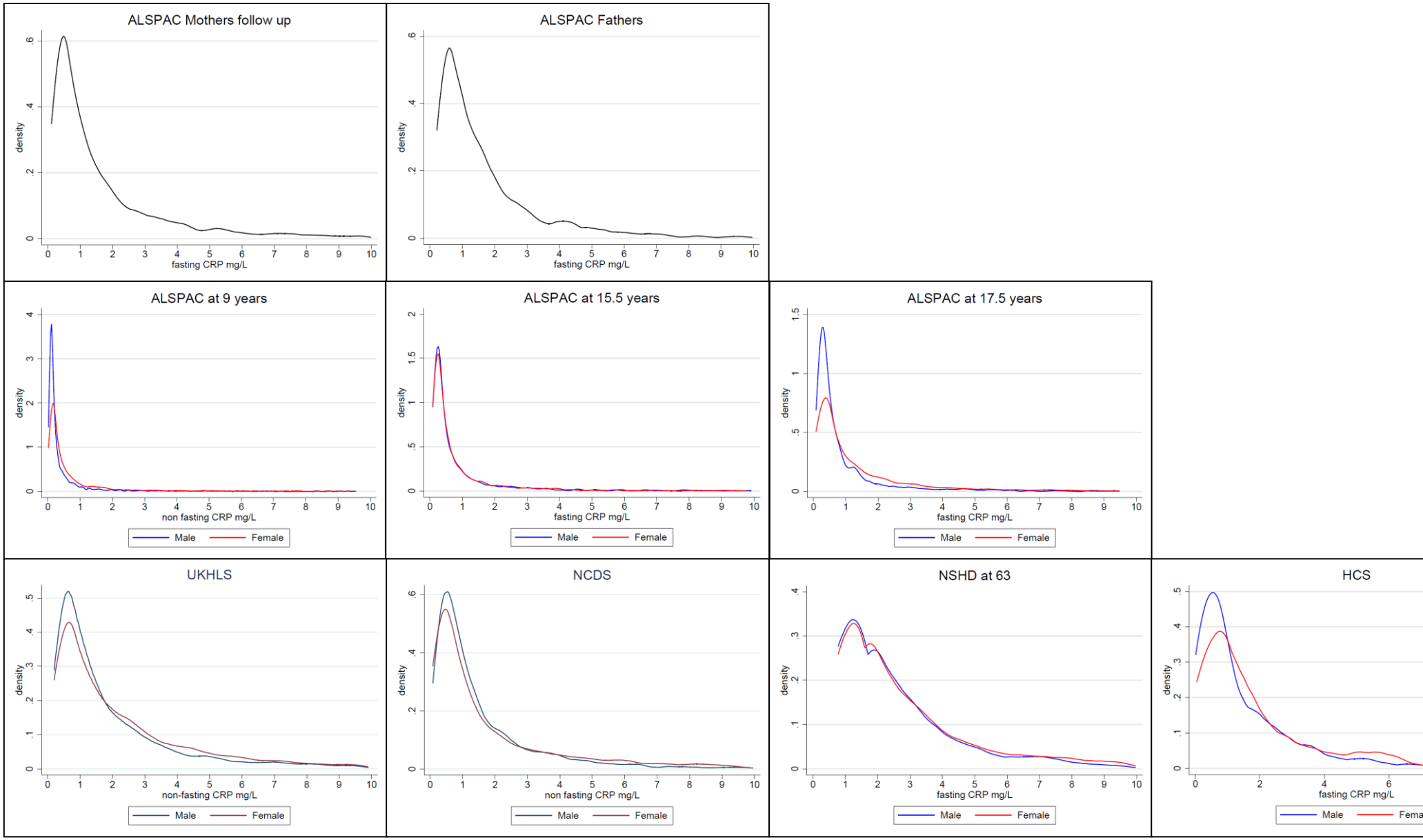


Figure B16. Distributions (Kernel density) of C-reactive protein (mg/L) by gender for each CLOSER study that measured CRP. Distributions were winsorised at 0.5% and 99.5%.



Fibrinogen

Fibrinogen has been measured in two CLOSER studies: UKHLS and NCDS (see **Table 2**).

What is it?

Fibrinogen is a glycoprotein. Through a series of enzymatic steps, it is converted into fibrin in the clotting process. Fibrinogen is also an "acute phase protein" and therefore reflects inflammatory processes.

What is its clinical significance?

Fibrinogen is a marker of inflammation and the body's ability to stop bleeding (haemostasis) implicated in the development of cardiovascular disease (CVD)[71].

How is it measured?

Fibrinogen should be measured from citrated plasma samples. As described in **Table A16** all studies that measured fibrinogen used the Clauss methodology to assay the analyte**.**

Fibrinogen is measured as described in **Table A17.**

Are there clinical cut points?

There are no established clinical cut points.

What should be considered in analyses?

Fibrinogen can be influenced by contraceptive drugs, hormone replacement therapy (BNF Chapter 6.4.1), anti-fibrinolytic drugs and haemostatics (BNF chapter 2.11). Moderate elevation in fibrinogen may be seen sometimes with pregnancy and smoking. Fibrinogen shows seasonal and diurnal variation.[72]

[71] Danesh J. et al. Plasma fibrinogen level and the risk of major cardiovascular diseases and nonvascular mortality. J. Am. Med. Assoc., 294 (2005), pp. 1799–1809

[72] Rudnicka, A. R. et al. Diurnal, Seasonal, and Blood-Processing Patterns in Levels of Circulating Fibrinogen, Fibrin D-Dimer, C-Reactive Protein, Tissue Plasminogen Activator, and von Willebrand Factor in a 45-Year-Old Population. *Circulation*, 2007;115:996-1003

Table A17. Fibrinogen (g/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	-	-	-	-
UKHLS	12 873	Modified Clauss thrombin clotting method on the IL-ACS-TOPS analyser	2.7 (0.6) 2.7 2.3-3.1	2.8 (0.6) 2.8 2.4-3.2
SWS	-	-	-	-
NCDS	7 683	Automated Clauss method in an MDA 180 coagulometer	2.9 (0.6) 2.8 2.5-3.2	3.0 (0.6) 3.0 2.6-3.4
NSHD	-	-	-	-
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A17. Crude plot of fibrinogen (g/L), median, by age for each CLOSER study that measured fibrinogen.

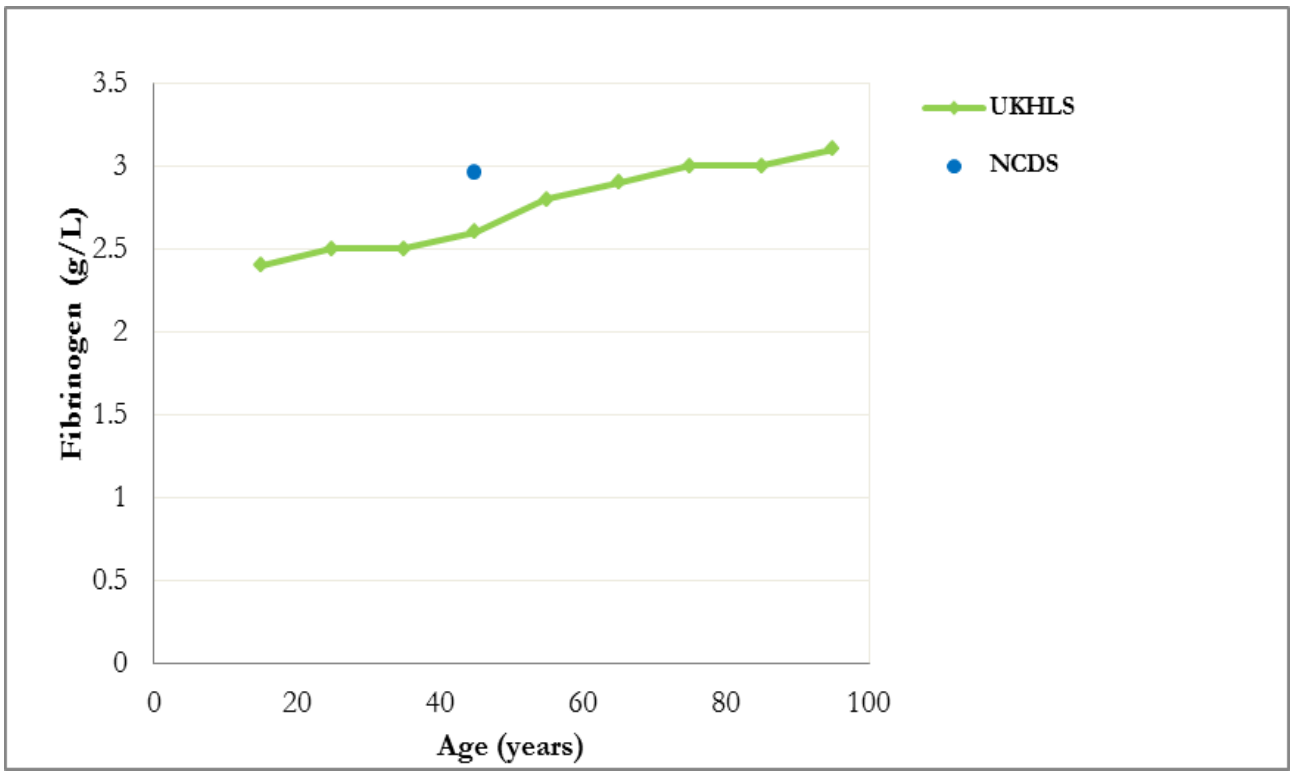
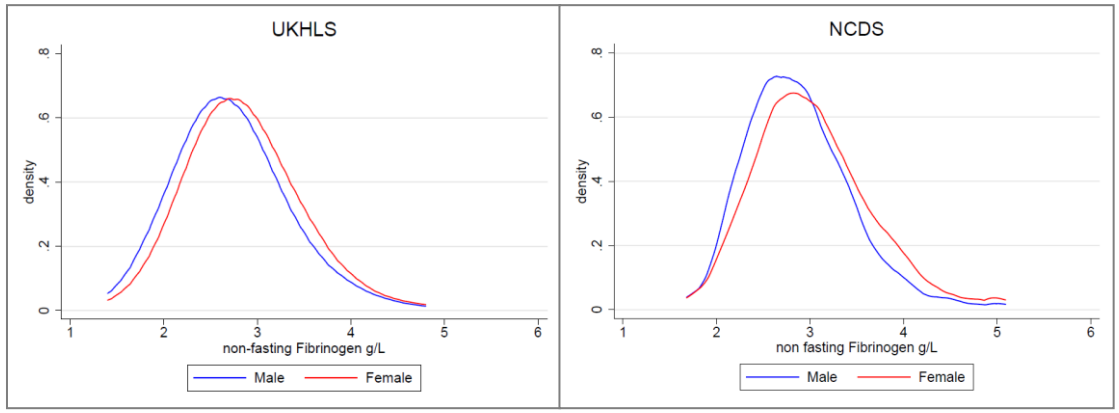


Figure B17. Distributions (Kernel density) of fibrinogen (g/L) by gender for each CLOSER study that measured fibrinogen. Distributions were winsorised at 0.5% and 99.5%.



Immunoglobulin E (IgE)

IgE has been measured in two CLOSER studies: ALSPAC and NCDS (see **Table 2**).

What is it?

Immunoglobulins are substances (proteins) made by the body's immune system in response to a number of substances such as bacteria, viruses, fungus, animal dander, or cancer cells. Antibodies attach to the foreign substances so the immune system can destroy them. Antibodies are specific to each type of foreign substance.

What is the clinical significance of IgE?

IgE antibody levels are often high in people with allergies and allergic conditions.

How is it measured?

IgE is measured as described in **Table A18.** NCDS and ALSPAC also measured allergen specific IgE; NCDS measured IgE response to house dust mites, mixed grasses and cat fur, while ALSPAC measured young children's IgE response to peanut, soya, egg, milk and fish.

Are there clinical cut points?

Reference values for IgE can vary by laboratory and therefore there are no clinical cut points.

What should be considered in analyses?

A number of conditions cause IgE to be high, including eczema, asthma, certain cancers, autoimmune diseases and atopic dermatitis.

Table A18. Immunoglobulin E, IgE (KU/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	8 mths	8 018	Fluoroimmunoassay using the Pharmacia UNICAP system	11.5 (17.2) 5.7 2.5-13.2	8.5 (12.3) 3.4 1.6-7.8
	12 mths	1 860		15.9(23.7) 6.3 2.6-16.1	11.3 (19.9) 4.7 2.3-10.1
	18 mths	5 088		18.2 (25.8) 6.8 2.8-21.3	16.0 (25.8) 5.4 2.2-14.5
	7 yrs	1 692		252.3 (508.7) 66 23-220	203 (421.1) 53.7 19.5-169
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	7 704		HYTEC enzyme immunoassay, with positive and negative controls	115.4 (253.2) 36 14-98	77.5 (196.5) 23 10-61
NSHD	-		-	-	-
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A18. Plot of IgE (KU/L), median, by age for each CLOSER study that measured IgE.

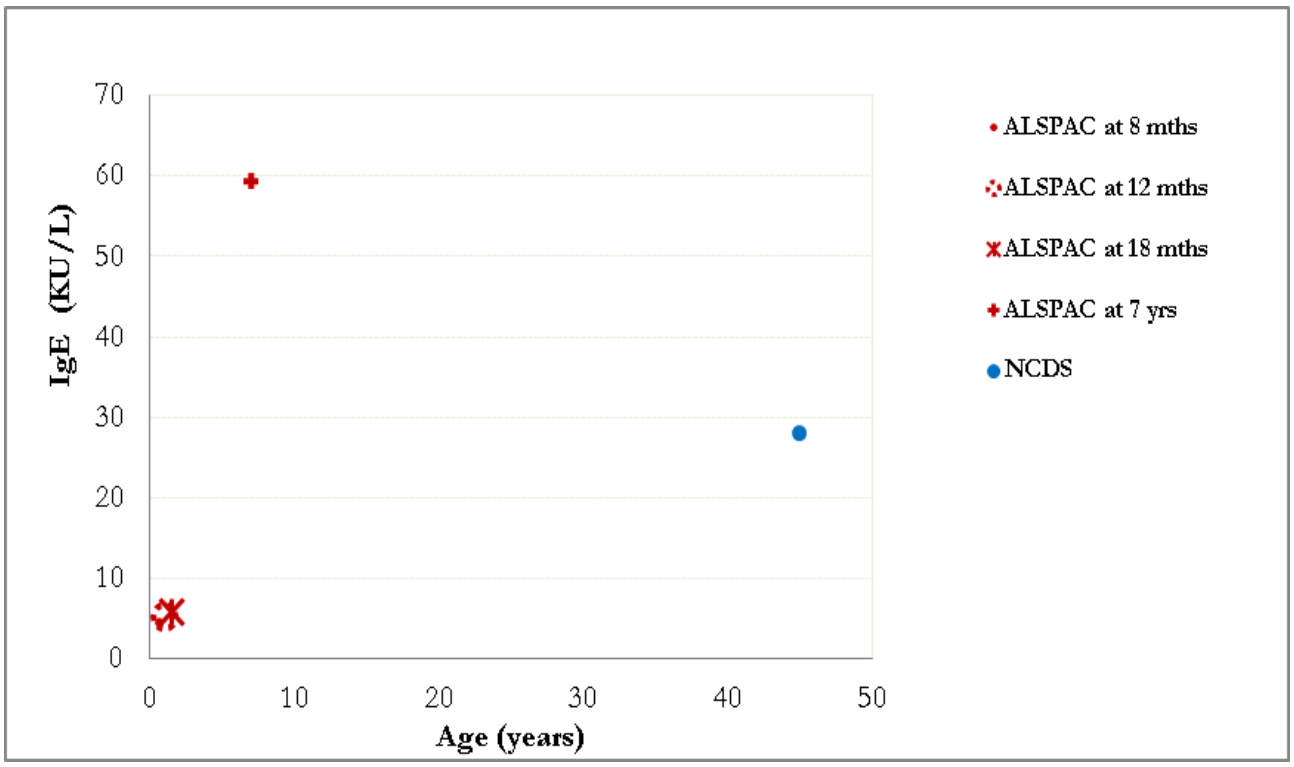
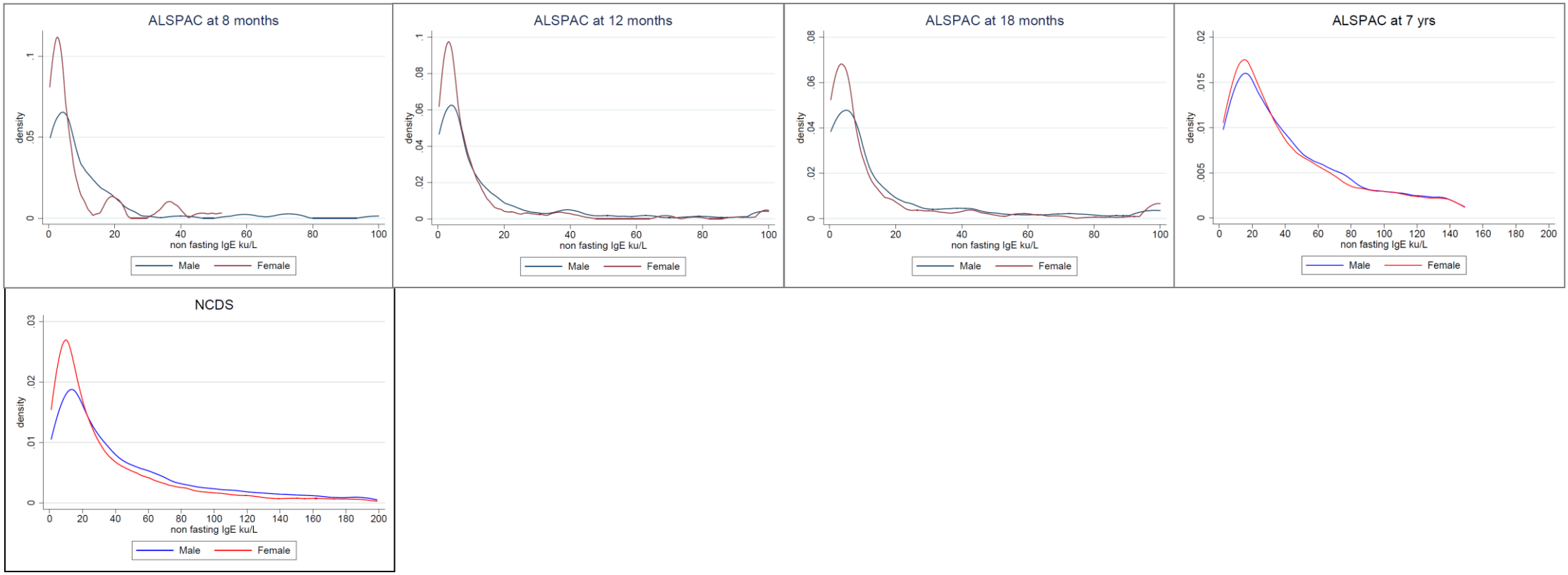


Figure B18. Distributions (Kernel density) of IgE (KU/L) by gender for each CLOSER study that measured IgE. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



Interleukin-6 (IL-6)

IL-6 has been measured in two CLOSER studies: ALSPAC and NSHD (see **Table 2**).

What is it?

IL-6 is an immune protein; it is produced in the body, wherever there is inflammation, either acute or chronic.

How is it measured?

All studies that measured IL-6 used an enzyme linked immunosorbent assay as described in **Table A19.**

What is the clinical significance of IL-6?

Helps to evaluate conditions such as diabetes and cardiovascular disease or conditions associated with inflammation such as lupus and rheumatoid arthritis; also helps to identify infections, such as sepsis.

Are there clinical cut points?

As the assay for IL-6 is not standardised across laboratories, there are no clinical cut points, thus, caution should be taken when comparing values across studies.

What should be considered in analyses?

There may be a diurnal variation in levels of IL-6 and analysts should check these in their analyses. IL-6 levels may be influenced by anti-inflammatory medications (BNF Chapter 10.1) and hormone medications BNF Chapter 6.4.1).

Table A19. Interleukin-6, IL-6 (pg/mL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	9 yrs	5 072	Enzyme-linked immunosorbent assay (ELISA)	1.2 (1.5) 0.7 0.4-1.2	1.4 (1.6) 0.9 0.6-1.6
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	2 052	High sensitivity ELISA	2.9 (2.8) 2.0 1.3-3.2	2.7 (2.8) 1.9 1.3-3.0
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A19. Plot of interleukin-6 (pg/mL), median, by age for each CLOSER study that measured IL-6.

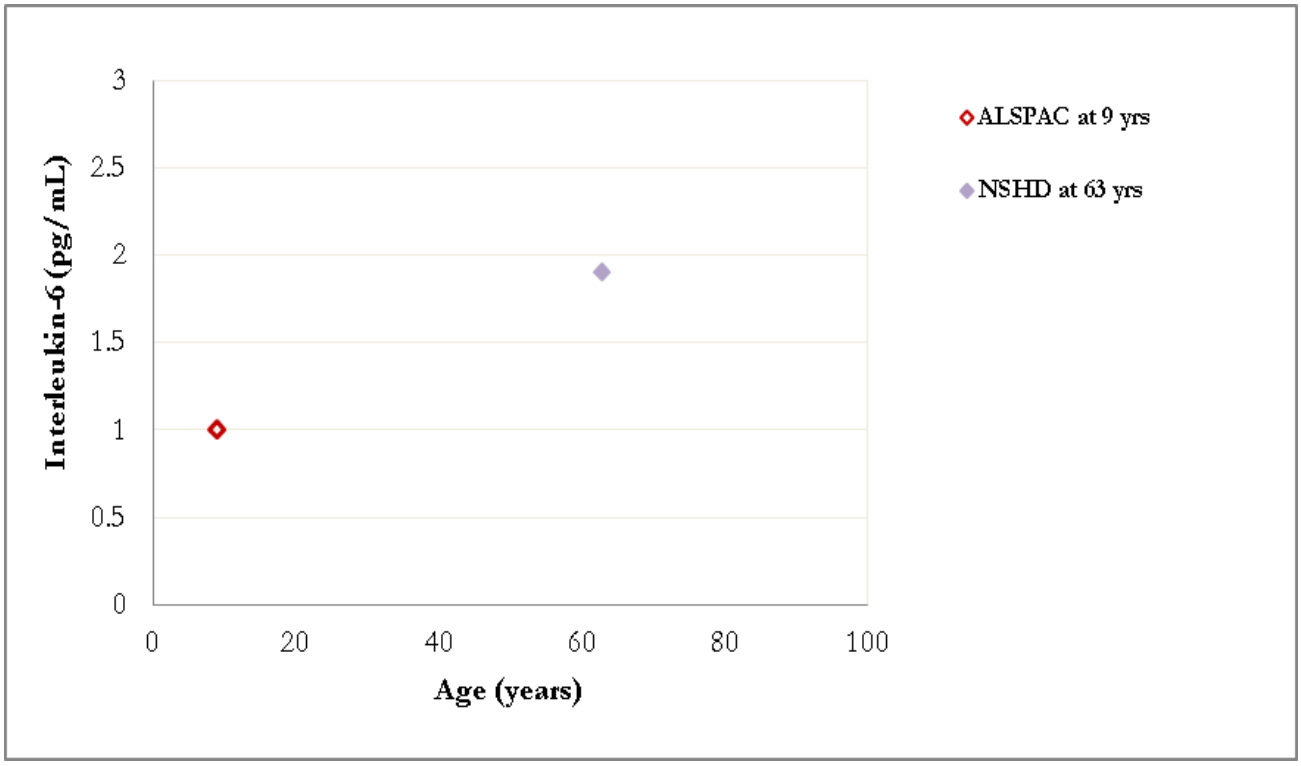
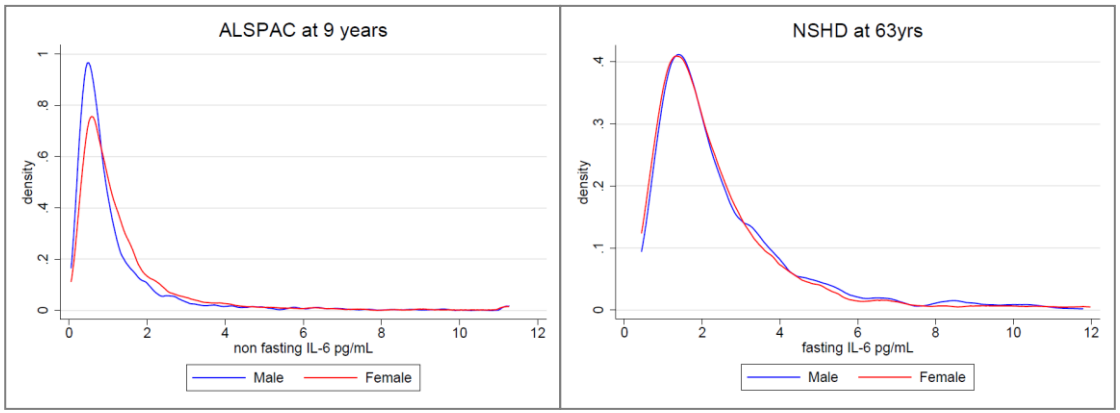


Figure B19. Distributions (Kernel density) of interleukin-6 (pg/mL) by gender for each CLOSER study that measured IL-6. Distributions were winsorised at 0.5% and 99.5%.



Red Cell Folate (RCF)

RCF has been measured in two CLOSER studies: SWS and NSHD (see **Table 2**).

What is it?

Red Cell Folate (or RBC Folate) is a measure of the body's store of the vitamin Folate also known as Folic Acid.

How is it measured?

RCF is measured as described in **Table A20.**

What is the clinical significance of RCF?

Folate performs several important functions in the body, including keeping the nervous system healthy. A deficiency in folate can cause a wide range of problems, including: extreme tiredness, pins and needles (paraesthesia), a sore and red tongue, mouth ulcers, muscle weakness, disturbed vision, psychological problems, which may include depression and confusion, problems with memory, understanding and judgement.

Are there clinical cut points?

There are no clinical cut points

What should be considered in analyses?

Certain medications, including anticonvulsants (BNF chapter 4.8) and proton pump inhibitors (PPIs, BNF chapter 1.3.5) can affect folate levels. Other factors affecting the levels of RCF in the body include age, pregnancy, smoking, oral contraceptive use and Vitamin B12 deficiency.

Table A20. Red Cell Folate, (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	-		-	-	-
UKHLS	-		-	-	-
SWS	Init EP†	8 619 1 736	Microparticle enzyme immunoassay	-	826.1 (368.4) 731.9 559.7-1003.8 1146.3 (462.6) 1067.3 804.4-1384.5
NCDS	-		-	-	-
NSHD	63 yrs	2 034	Competitive protein binding chemiluminescent method	316.4 (96.5) 313 257-372	367.6 (102.0) 359.0 305.0-433.0
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar, including outliers**

†EP: Approximate gestation age of 11 weeks; SWS measured RCF in late pregnancy (LP) for 89 women only.

Figure A20. Plot of red cell folate (nmol/L), median, by age for each CLOSER study that measured RCF.

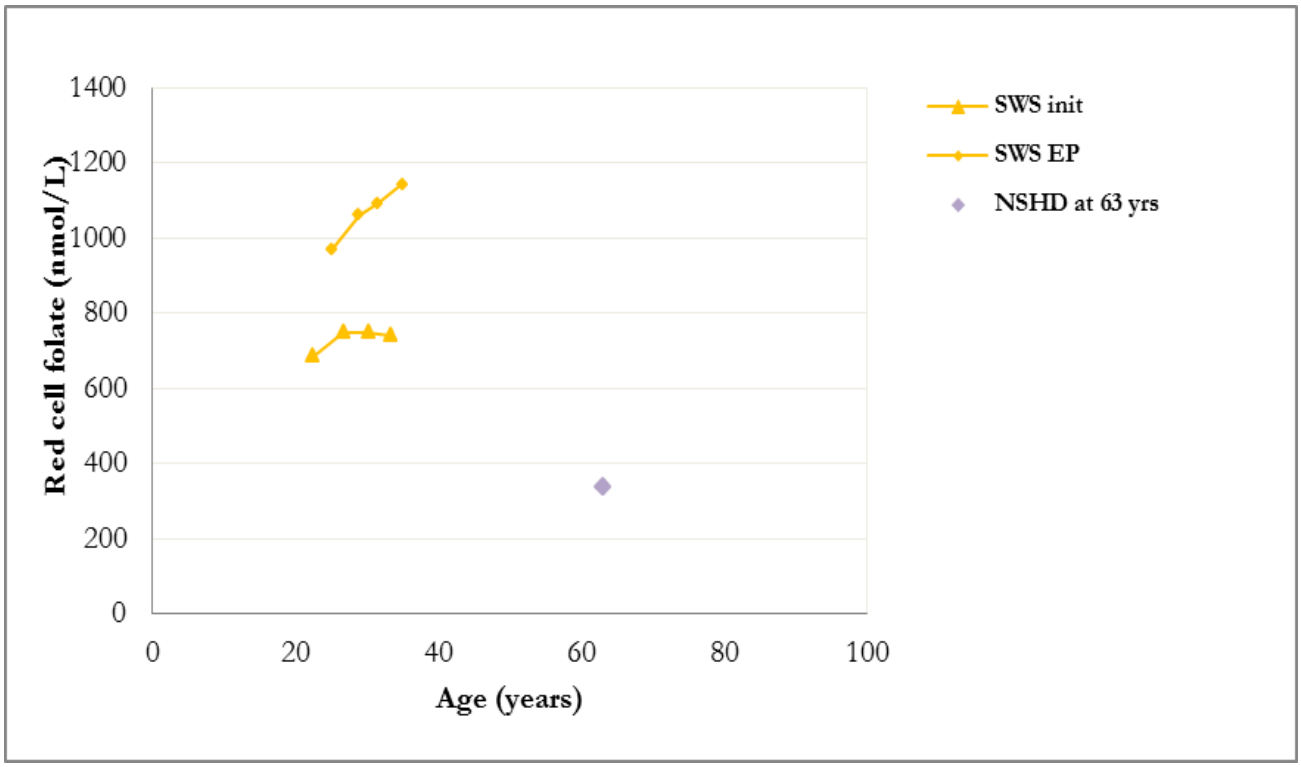
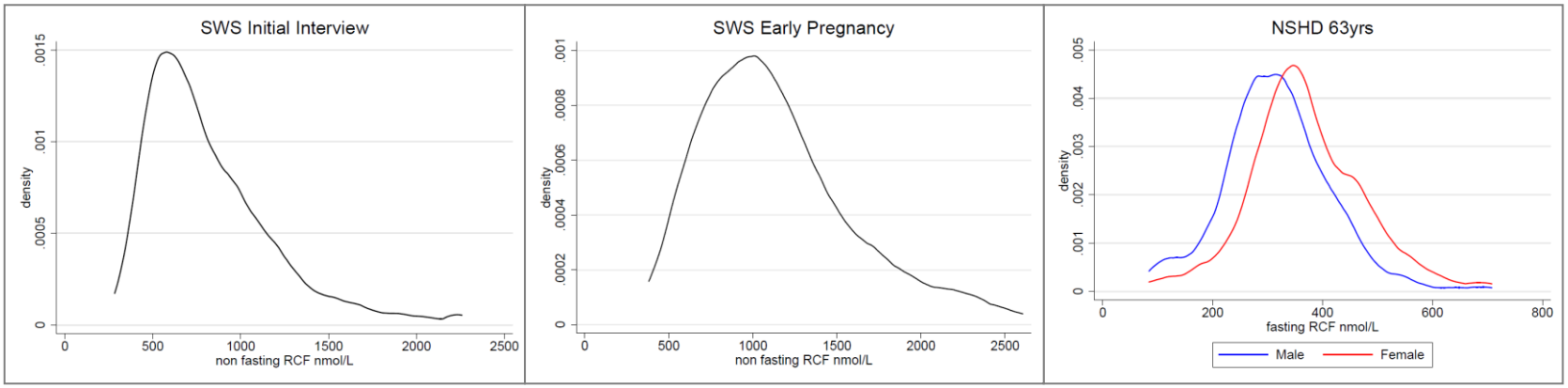


Figure B20. Distributions (Kernel density) of red cell folate (nmol/L) by gender for each CLOSER study that measured RCF. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



Tissue Plasminogen Activator (TPA)

TPA has been measured in two CLOSER studies: NCDS and NSHD (see **Table 2**).

What is it?

TPA is a protein involved in the breakdown of blood clots.

How is it measured?

TPA is measured using enzyme linked immunosorbent assays as described in **Table A21.**
Typically, samples should be taken from a rested, fasting subject in the morning with no consumption of cigarettes or alcohol in the hour beforehand.

What is the clinical significance of TPA?

The Tissue Plasminogen Activator assay is used to detect disorders of the fibrinolytic system such as Thrombosis, Pulmonary embolism, acute myocardial infarction and Stroke

Are there clinical cut points?

Normal values are as follows:

Adult: 1.6 to 13.0 ng/mL
Pregnancy Trimester One: 1.8 to 6.0 ng/mL
Pregnancy Trimester Two: 2.4 to 6.6 ng/mL
Pregnancy Trimester Three: 3.3 to 9.2 ng/mL

What should be considered in analyses?

It has been shown that there is significant diurnal and seasonal variation in TPA levels and these should be taken into account when considering epidemiological analysis[57]. TPA levels show diurnal and seasonal variation and are particularly sensitive to delays in sample processing.[73] TPA levels rise during pregnancy.

[73] Rudnicka A.R. et al. Diurnal, Seasonal, and Blood-Processing Patterns in Levels of Circulating Fibrinogen, Fibrin D-Dimer, C-Reactive Protein, Tissue Plasminogen Activator, and von Willebrand Factor in a 45-Year-Old Population. *Circulation*, 2007;115:996-1003

Table A21. Tissue plasminogen activator, TPA (ng/mL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	-		-	-	-
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	7 692		ELISA assays (double-antibody sandwich)	5.9 (2.8) 5.5 3.8-7.5	4.5 (2.5) 3.9 2.7-5.7
NSHD	63 yrs	1 798	ELISA	10.6 (5.6) 9.9 6.8-12.7	9.3 (5.1) 8.5 6.0-11.5
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A21. Plot of tissue plasminogen activator (ng/mL), median, by age for each CLOSER study that measured TPA.

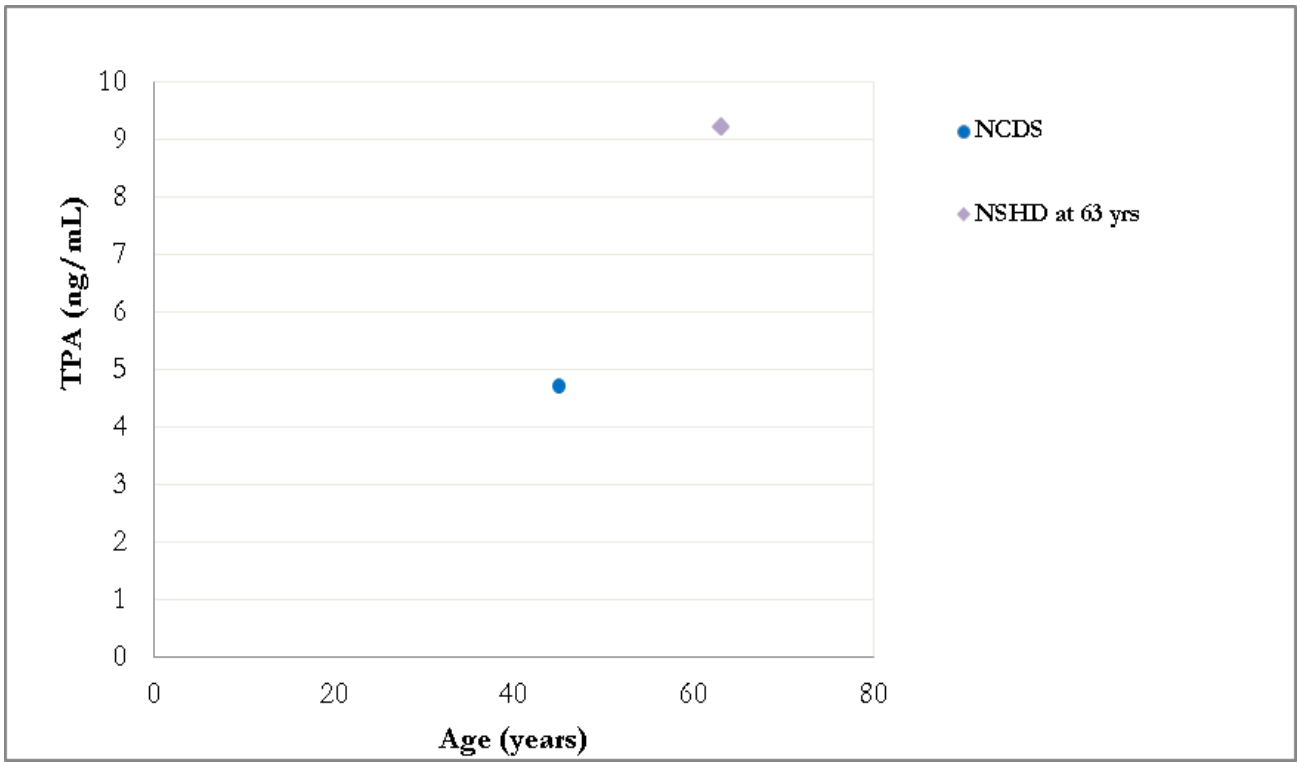
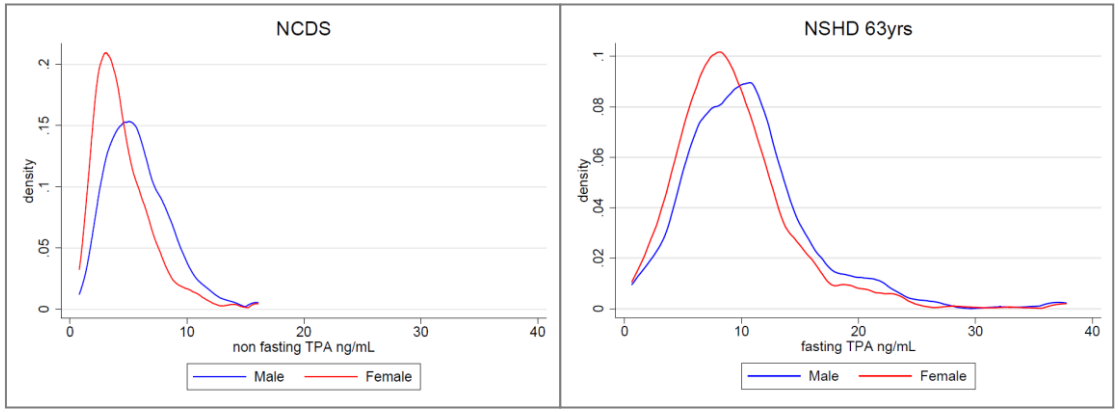


Figure B21. Distributions (Kernel density) of tissue plasminogen activator (ng/mL) by gender for each CLOSER study that measured TPA. Distributions were winsorised at 0.5% and 99.5%.



Von Willebrand Factor (VWF)

Von Willebrand Factor is measured in two of the CLOSER studies: NCDS and NSHD (see **Table 2**).

What is it?

VWF is a protein, one of several components of the coagulation system that work together to stop bleeding and form a stable blood clot. VWF is produced by megakaryocytes and by the endothelial cells that line blood vessels. It is released by platelets and endothelial cells as needed.

What is the clinical significance of VWF?

VWF plays a major role in blood coagulation. Therefore, VWF deficiency or dysfunction (von Willebrand disease) leads to a bleeding tendency. Unusual levels may be associated with a variety of bleeding disorders but are not considered diagnostic.

How is it measured?

VWF is measured as described in **Table A22**.

Are there clinical cut points?

In the general population, the mean level of plasma VWF is 100 IU/dL, with a normal reference range between 50 and 200 IU/dL.

What should be considered in analyses?

The use of oestrogen or oral contraceptives may falsely elevate VWF. Stress, very recent exercise, acute or chronic illness, inflammation and pregnancy can also have an impact on the levels of VWF. A person's ABO blood type affects VWF concentrations; people with type O blood have VWF levels that are up to 25% lower than those with other blood types. VWF shows diurnal variation[74].

[74] Rudnicka A.R. Diurnal, Seasonal, and Blood-Processing Patterns in Levels of Circulating Fibrinogen, Fibrin D-Dimer, C-Reactive Protein, Tissue Plasminogen Activator, and von Willebrand Factor in a 45-Year-Old Population. *Circulation*, 2007;115:996-1003

Table A22. Von Willebrand Factor, VWF (IU/dL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	-		-	-	-
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	7 686		Enzyme-linked immunosorbent assays employing a double sandwich technique on citrate plasma	123.7 (40.3) 120.0 94.0-147.0	121.0 (40.1) 115.0 91.0-144.0
NSHD	63 yrs	1 794	DELFIA two-site fluoroimmunometric assay	136.1 (63.6) 129.0 90.0-174.0	136.6 (63.5) 130.0 92.0-172.0
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**.

Figure A22. Crude plot of von willebrand factor (IU/dL), median, by age for each CLOSER study that measured VWF.

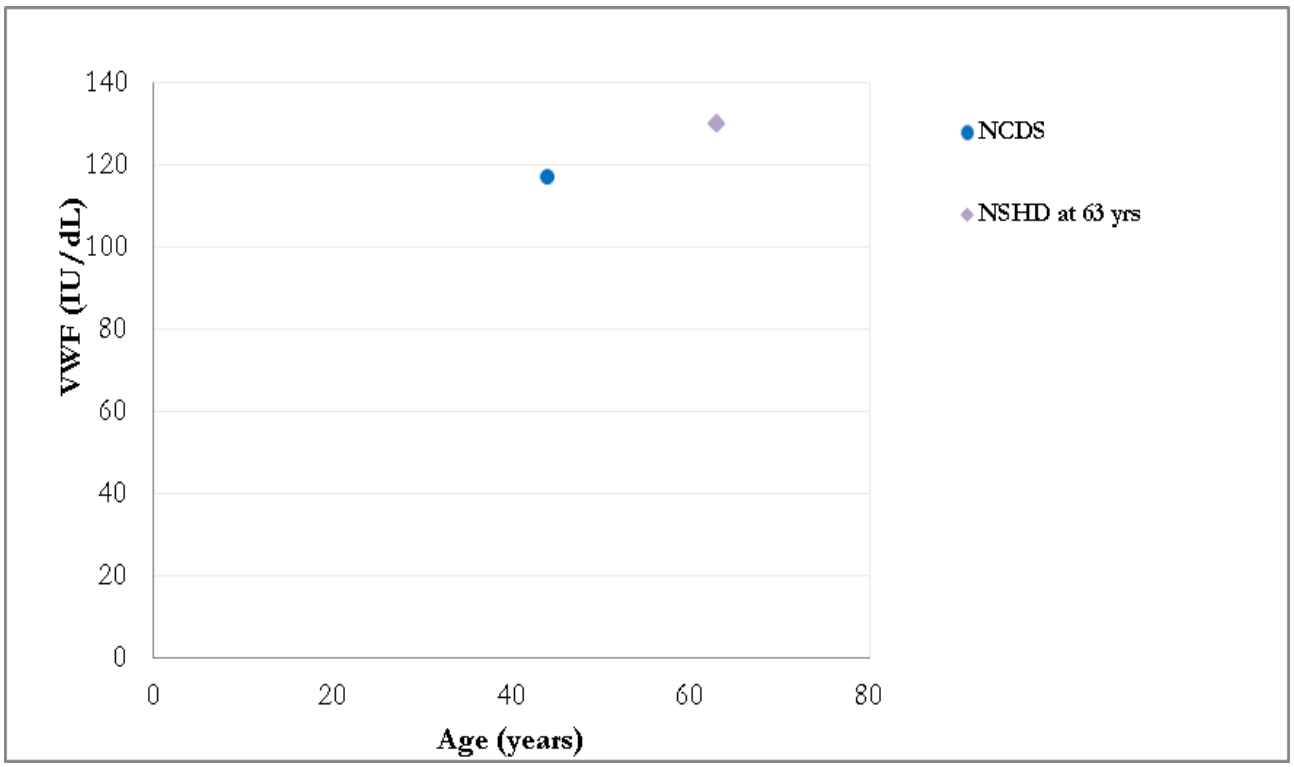
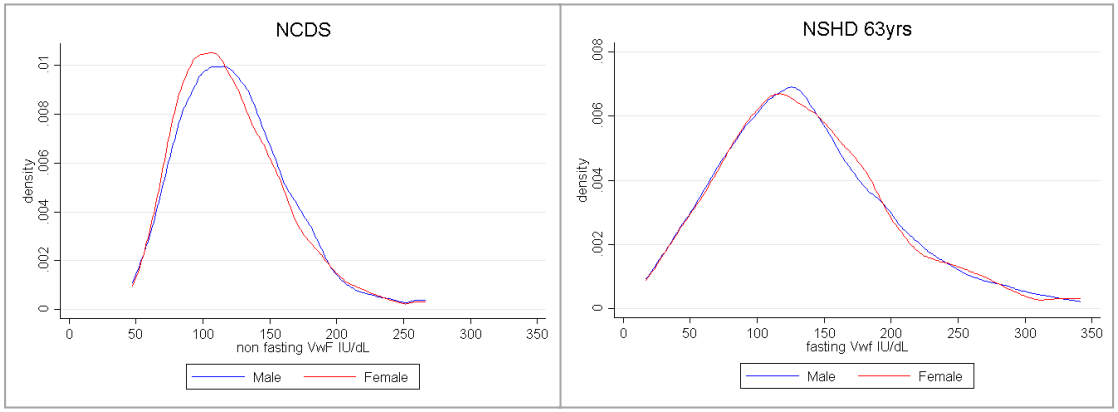


Figure B22. Distributions (Kernel density) of von willebrand factor (IU/dL) by gender for each CLOSER study that measured VWF. Distributions were winsorised at 0.5% and 99.5%.



Platelet count

Platelets have been measured in two CLOSER studies: SWS and NSHD (see Table 2).

What is it?

Platelets are parts of the blood system that help the blood clot. A platelet count is used to measure how many platelets there are in a blood sample. They are smaller than red or white blood cells.

How is it measured?

Platelet count is measured as described in **Table 23**.

What is the clinical significance of Platelet count?

The number of platelets in the blood system can be affected by many diseases. Platelets may be counted to monitor or diagnose diseases, or to look for the cause of too much bleeding or clotting.

Are there clinical cut points?

An average normal range of Platelet count is between $150x10^9$ and $400x10^9$ platelets per litre. Numbers may vary slightly but counts that are higher or lower than this range are classified as abnormal.

What should be considered in analyses?

Abnormal Platelet count may be due to an ongoing health condition, drugs or medications. Drugs that interfere with platelet count are aspirins, ibuprofen, non-steroidal anti-inflammatory drugs (NSAIDs, BNF 10.1.1) or other medications that contain these, tricyclic antidepressants (BNF 4.3), antihistamines (BNF 3.4.1) and some antibiotics. Low platelet count (thrombocytopenia) is observed when undergoing cancer treatments (chemotherapy and radiation therapies) and due to the presence of autoimmune disorders or chronic conditions such as kidney failure and Myelodysplastic syndrome (MDS). High platelet count (thrombocytosis) can be due to anaemia, infections, surgery, cancer and bone marrow disease.

Table A23. Platelet count (x10⁹/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY		Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC		-	-	-	-
UKHLS		-	-	-	-
SWS	Init	8 708	Automated method	-	263.7 (58.2) 259 222-299
	EP†	1 979			253.1 (50.8) 249 217-286
	LP‡	2 308			245.8 (60.9) 238 204-281
NCDS		-	-	-	-
NSHD	63 yrs	2 053	In-house methodology	220.8 (52.7) 218 184-253	249.4 (54.2) 244 213-285
HCS		-	-	-	-

Figure A23. Crude plot of platelet count (x10^9/L), median, by age for each CLOSER study that measured platelet count.

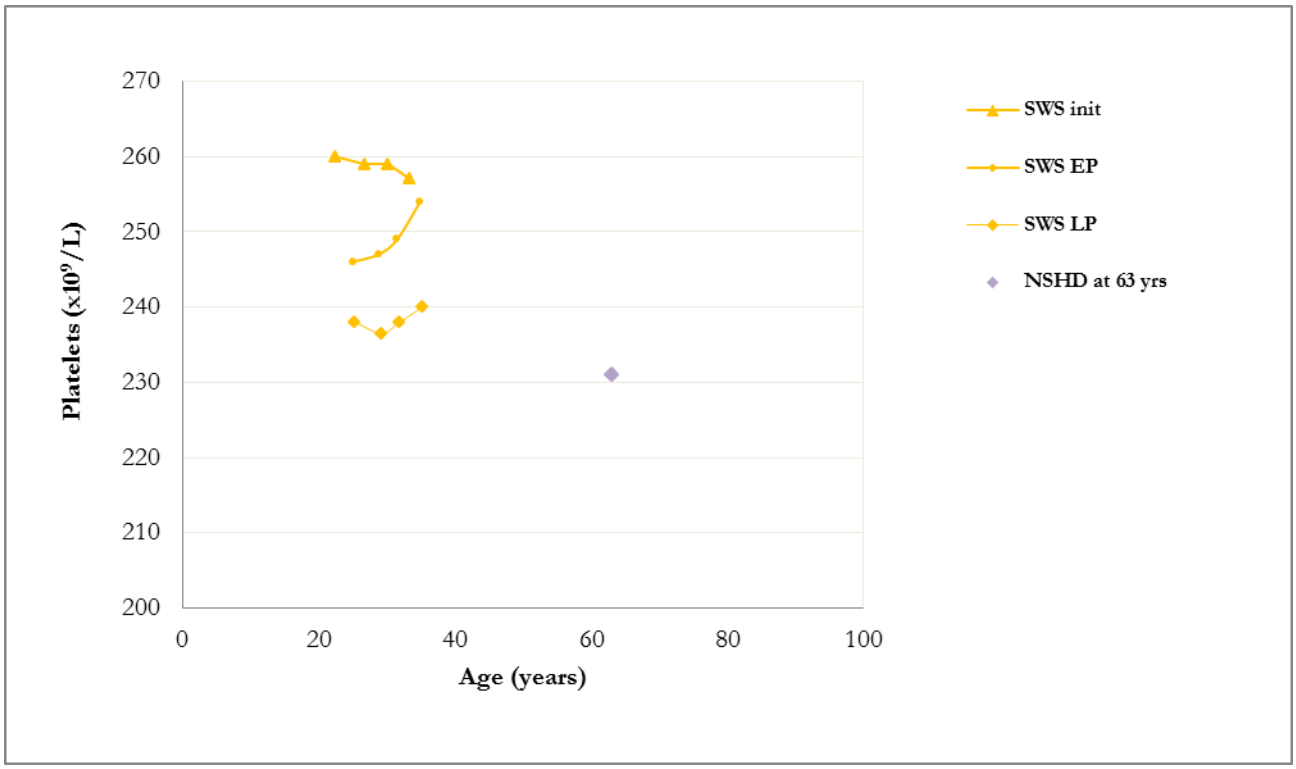
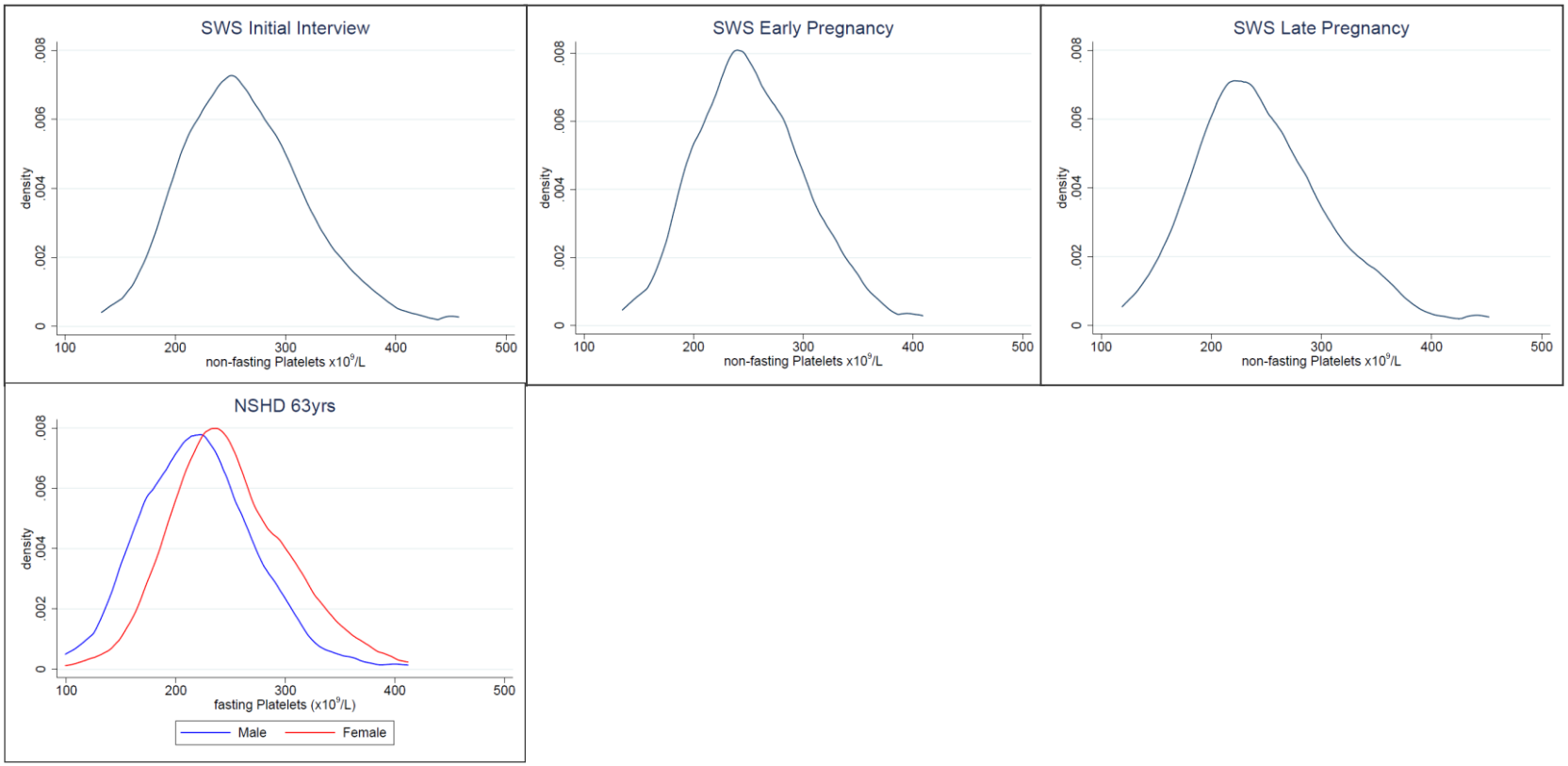


Figure B23. Distributions (Kernel density) of Platelet count (x10^9/L) by gender for each CLOSER study that measured platelet count. Distributions were winsorised at 0.5% and 99.5%.



Red Blood Cell (RBC) count

RBC count has been measured in two CLOSER studies: SWS and NSHD (see **Table 2**).

What is it?

RBC count, also known as erythrocyte count, is the actual number of red blood cells in a person's sample of blood.
RBC count is part of a routine medical exam; when individuals have signs and symptoms that may be related to a condition that affects blood cells.

How is it measured?

Red blood cells are measured as described in **Table 24.**

What is the clinical significance of RBC counts?

RBCs contain haemoglobin, which carries oxygen to the body's tissues. The number of RBCs in the blood can affect how much oxygen the tissues receive. Body tissues need oxygen to function.
There are various RBC indices which provide information on the physical characteristics of the RBCs, these are:

- Mean corpuscular volume (MCV): is a measurement of the average size of a single red blood cell.
- Mean corpuscular haemoglobin (MCH): is a calculation of the average amount of haemoglobin inside a single red blood cell.
- Mean corpuscular haemoglobin concentration (MCHC): is a calculation of the average concentration of haemoglobin inside a single red blood cell.
- Red cell distribution width (RBCDW): is a calculation of the variation in the size of RBCs.

Some of these indices have been measured in CLOSER studies; SWS measured MCV, MCH, MCHC and RBCDW; NSHD at 63 yrs, has measured MCV and MCH (see following **Table 24a**).

Are there clinical cut points?
Age and gender affect the number of red blood cells in the blood:

•The normal RBC range for men is 4.7 to 6.1 million cells per microliter (mcL).
•The normal RBC range for women who aren't pregnant is 4.2 to 5.4 million mcL.
•The normal RBC range for children is 4.0 to 5.5 million mcL.

These ranges may vary depending on the laboratory.

What should be considered in analyses?

RBC counts vary according to age and gender. Women tend to have lower RBC counts than men, and levels tend to decrease with age. A high RBC count may indicate congenital heart disease, dehydration, obstructive lung disease, or bone marrow over-production. A low RBC count may indicate anaemia, bleeding, kidney disease, bone marrow failure (for instance, from radiation or a tumour), malnutrition, or other causes. A low count may also indicate nutritional deficiencies of iron, folate and vitamin B12. A decrease in red blood cells is seen during

pregnancy as a result of normal body fluid increases that dilute them. Living at high altitudes causes an increase in RBC counts, as the body's response to the decreased oxygen available at these heights.

Certain drugs like gentamicin (antibiotic) and methyldopa (high blood pressure medication) can increase the RBC count. Performance-enhancing drugs like protein injections and anabolic steroids can increase RBCs. Certain drugs can lower the RBC count, especially: chemotherapy drugs, chloramphenicol, quinidine and hydantoins.

Table A24. Red Blood Cell, RBC count (x10^{12}/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY		Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC		-	-	-	-
UKHLS		-	-	-	-
SWS	Init	7 749	Automated method	-	4.3 (0.3) 4.3 4.1-4.5
	EP†	1 981			4.2 (0.3) 4.2 4.0-4.4
	LP‡	2 309			3.9 (0.3) 3.9 3.7-4.1
NCDS		-	-	-	-
NSHD	63 yrs	2 061	In-house methodology	4.8 (0.4) 4.8 4.6-5.1	4.5 (0.3) 4.5 4.2-4.7
HCS		-	-	-	-

Table 24a. Red Blood Cell indices (MCV and MCH): participants (N), mean (x10^{15} counts per L of blood) and standard deviation of the mean (SD) for each RBC index measured in NSHD at 63 yrs and SWS.

	NSHD at 63		SWS		
	M	**F**	**Initial interview**	**Early pregnancy**	**Late pregnancy**
	N, mean (SD)	**N, mean (SD)**	**N, mean (SD)**	**N, mean (SD)**	**N, mean (SD)**
MCV (mean corpuscular volume)	999, 90.4 (4.7)	1 061, 90.1 (4.4)	8 709, 87.9 (4.4)	1 981, 88.2 (4.0)	2 309, 88.4 (4.9)
MCH (mean corpuscular haemoglobin)	998, 30.9 (2.0)	1 060, 30.4 (1.7)	8 707, 30.1 (1.8)	1 981, 30.5 (1.6)	2 309, 29.8 (2.1)

Figure A24. Crude plot of Red Blood cell count (x10^{12}/L), median, by age for each CLOSER study that measured RBC count.

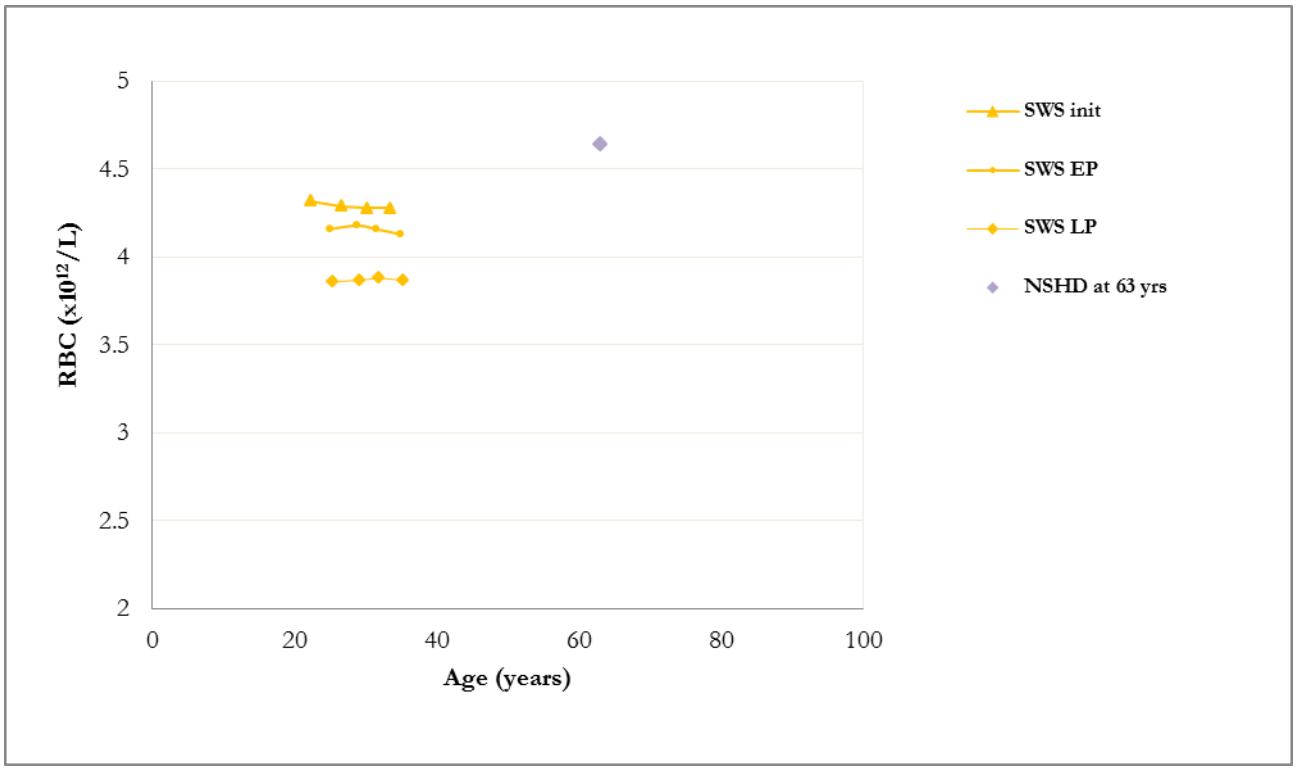
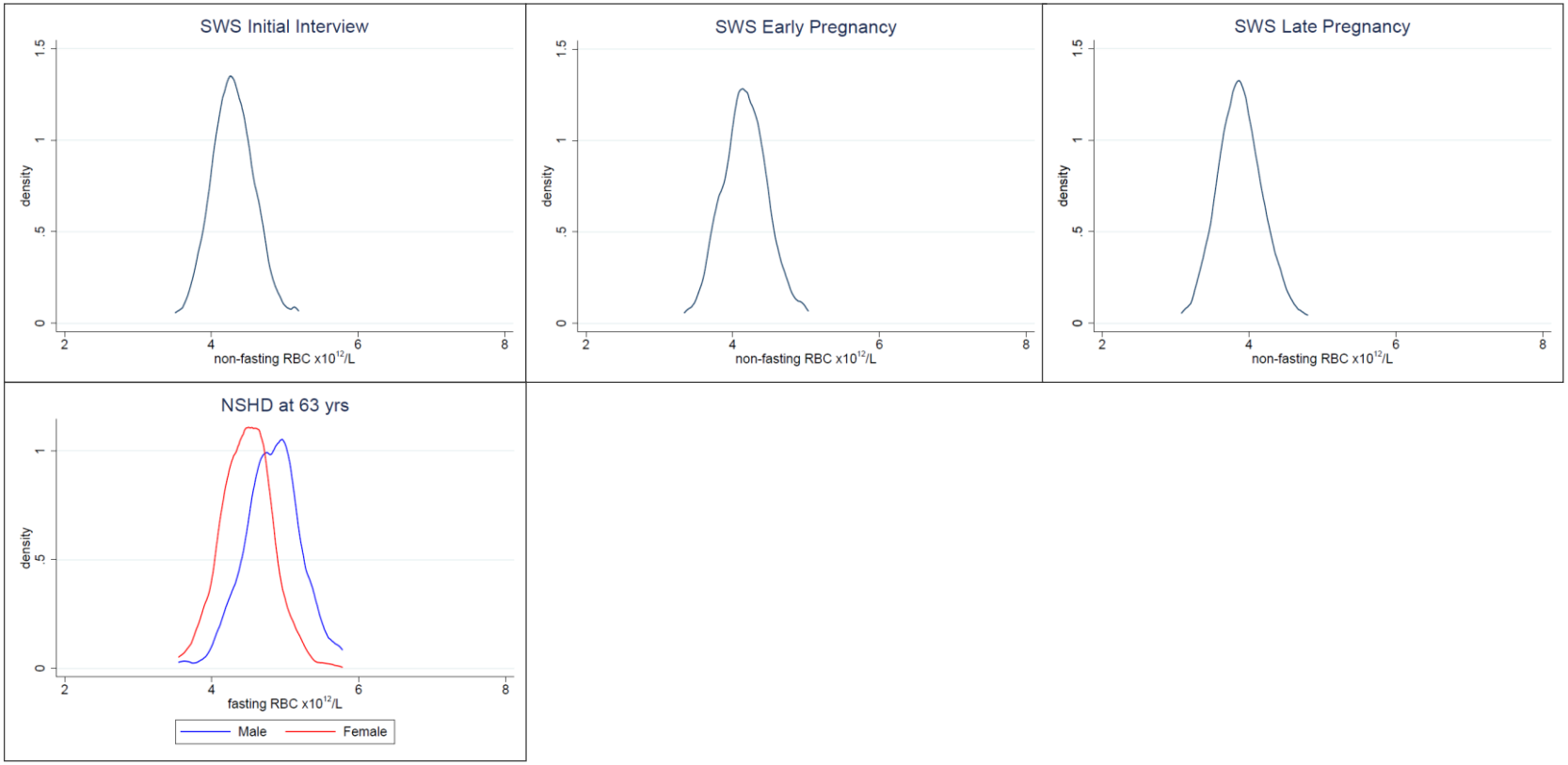


Figure B24. Distributions (Kernel density) of Red Blood cell count (x10^{12}/L) by gender for each CLOSER study that measured platelet count. Distributions were winsorised at 0.5% and 99.5%.



White Blood Cell (WBC) count

WBC count has been measured in two CLOSER studies: SWS and NSHD (see **Table 2**).

What is it?

White blood cells are an important component of the blood system, which is also made up of red blood cells, platelets, and plasma. WBC however account for only 1% of the total blood volume.

How is it measured?

White blood cells are measured as described in **Table A25.**

What is the clinical significance of WBC?

White-blood cells help fight infections by attacking bacteria, viruses, and germs that invade the body. A WBC count can detect hidden infections and undiagnosed medical conditions.

There are five major types of white blood cells: Neutrophils, Lymphocytes, Eosinophils, Monocytes and Basophils; these have been measured in two CLOSER studies, SWS and NSHD at 63 yrs. (see following **Table A25a**).

Are there clinical cut points?

An average normal range of WBC counts is between $4.5x10^9$ and $10x10^9$ white blood cells per litre. Numbers that are higher or lower than this range are classified as abnormal. As with all biomarkers, age can also affect the number of white blood cells, with infants having a higher count than adults.

What should be considered in analyses?

Certain medications can interfere with lab results and either lower or increase the WBC count including: corticosteroids (BNF chapter 6.3), quinidine (BNF chapter 9.1.5) heparin BNF chapter 2.8.1), clozapine (BNF chapter 4.2.1), antibiotics, antihistamines (BNF chapter 3.4.1), diuretics (BNF chapter 2.2), anticonvulsants (BNF chapter 4.8), sulphonamide and chemotherapy medication. Increased WBC levels may be seen during the late stages of pregnancy.

Table A25. White Blood Cell, WBC, count (x10^9/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY		Valid*, N	Method of Measurement	Mean (sd), Median, IQR Males	Mean (sd), Median, IQR Females
ALSPAC		-	-	-	-
UKHLS		-	-	-	-
SWS	Init	8 709	Automated method	-	7.4 (1.8) 7.2 6.2-8.5
	EP†	1 981			9.3 (2.1) 9.1 7.8-10.6
	LP‡	2 309			10.4 (2.4) 10.2 8.8-11.7
NCDS		-	-	-	-
NSHD	63 yrs	2 062	In-house methodology	6.3 (1.7) 6.0 5.1-7.2	5.9 (1.6) 5.7 4.8-6.7
HCS		-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

†EP: Approximate gestation age is 11 weeks

‡LP: Approximate gestation age is 34 weeks.

Table A25a. WBC types: participants (N), mean (x10^9 counts per L of blood) and standard deviation of the mean (SD) for each type of white blood cell measured in NSHD at 63 yrs and SWS

WBC type	NSHD at 63 yrs		SWS		
	M	F	Initial interview	Early pregnancy	Late pregnancy
	N, mean (SD)	N, mean (SD)	N, mean (SD)	N, mean (SD)	N, mean (SD)
LYMPHOCYTES	999, 1.7 (0.5)	1 061, 1.8 (0.6)	8 708, 2.3 (0.7)	1 981, 2.0 (0.6)	2 308, 1.8 (0.5)
MONOCYTES	999, 0.5 (0.2)	1 060, 0.4 (0.1)	8 708, 0.5 (0.2)	1 981, 0.6 (0.2)	2 307, 0.7 (0.2)
EOSINOPHILS	998, 0.2 (0.1)	1 056, 0.2 (0.1)	8 707, 0.2 (0.2)	1 981, 0.2 (0.1)	2 308, 0.1 (0.1)
NEUTROPHYLES	999, 3.8 (1.3)	1 063, 3.4 (1.3)	8 708, 4.3 (1.4)	1 981, 6.5 (1.7)	2 308, 7.7 (2.0)
BASOPHILS	902, 0.04 (0.03)	951, 0.04 (0.03)	8 708, 0.04 (0.05)	1 981, 0.04 (0.05)	2 308, 0.03 (0.06)

Figure A25. Crude plot of white blood cells count (x10^9/L), median, by age for each CLOSER study that measured WBC.

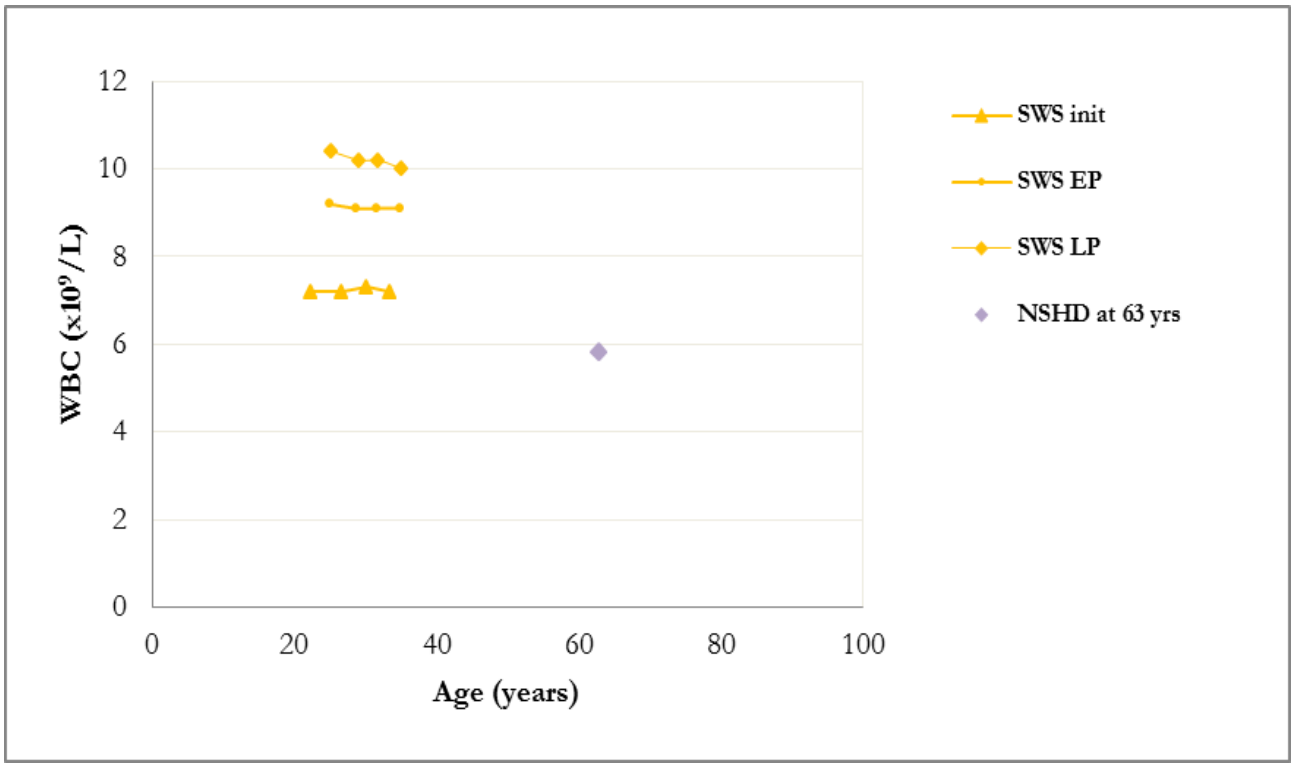
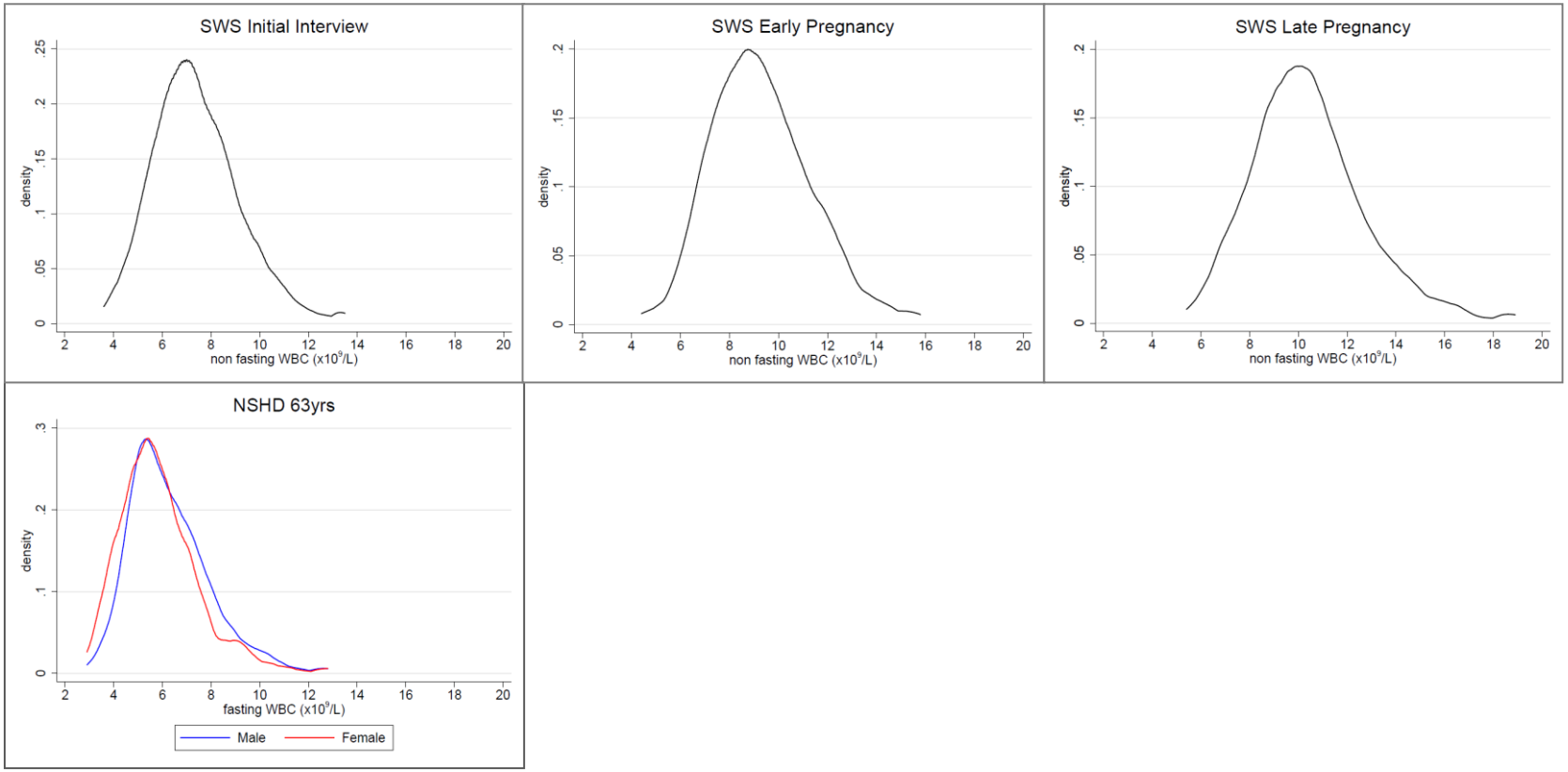


Figure B25. Distributions (Kernel density) of white blood cells count (x10^9/L) by gender for each CLOSER study that measured WBC. Distributions were winsorised at 0.5% and 99.5%.



3.6 Neuroendocrine Biomarkers

Cortisol

DHEAS

Insulin like Growth Factor-1 (IGF-1)

Insulin like Growth Factor-2 (IGF-2)

Insulin like Growth Factor Binding Protein-3 (IGFBP3)

Sex Hormone Binding Globulin (SHBG)

Testosterone

3.6.1 Association of Neuro-endocrine markers and the social environment

Neuroendocrine markers have been proposed as mediators of the association of the social environment as health and the biological mediators by which the environment 'gets under the skin'. Cortisol shows strong diurnal patterns across the day, making it difficult to assess levels in large scale epidemiological settings[75]. However, CLOSER studies are well represented in studies that have tried to assess cortisol levels. Cortisol patterns have been assessed as the decline in cortisol across the day and also the rise in cortisol levels after waking – the so-called cortisol awakening response, CAR. Findings suggests that cortisol decline across the day is associated with mortality[76]. Studies suggest that cortisol levels are associated with measures of social position[59] and marital status[77].

Other neuroendocrine markers have been associated with mortality, for example, low levels of the end product of hypothalamic-pituitary-growth hormone axis, IGF-1[78] and the steroid DHEAs[79]. Other markers of neuroendocrine function and steroids are shown to be socially patterned including IGF-1 in NSHD[80], NCDS[81] and testosterone in NSHD[63].

[75]Adam E.K., Kumari M. Assessing salivary cortisol in large-scale, epidemiological research.Psychoneuroendocrinology. 2009 Nov;34(10):1423-3

[76]Kumari M. et al. Association of diurnal patterns in salivary cortisol with all-cause and cardiovascular mortality: findings from the Whitehall II study. J Clin Endocrinol Metab. 2011 May;96(5):1478-85.

[77] Stafford M. et al Social isolation and diurnal cortisol patterns in an ageing cohort. Psychoneuroendocrinology. 2013 Nov;38(11):2737-45

[78] Goldman N. et al. Predicting Mortality From Clinical and Nonclinical Biomarkers. J Gerontol A Biol Sci Med Sci (2006) 61 (10): 1070-1074

[62] Glei D.A., Goldman N. Dehydroepiandrosterone sulfate (DHEAS) and risk for mortality among older Taiwanese. Ann Epidemiol. 2006 Jul;16(7):510-5.

[80] Bann D. et al. Socioeconomic conditions across life related to multiple measures of the endocrine system in older adults: Longitudinal findings from a British birth cohort study. Soc Sci Med. 2015 Dec;147:190-9.

[81] Kumari M. et al. Social differences in insulin-like growth factor-1: findings from a British birth cohort. Ann Epidemiol. 2008 Aug;18(8):664-70.

Cortisol

Salivary cortisol has been measured in four CLOSER studies: ALSPAC, NCDS, NSHD and HCS.

Blood cortisol has been measured in ALSPAC and HCS (see **Table 2**).

What is it?

Cortisol is a steroid hormone made from the adrenal gland. It is released in response to stress.

What is the clinical significance of cortisol?

Cortisol is associated with a number of disease outcomes but it is not used in clinical practice.

How is it measured?

Cortisol varies significantly throughout the day and is therefore measured in a number of ways. To assess this diurnal variation, cortisol can be measured from saliva samples. Cortisol is measured as described in **Table A26** and **Table A27**.

Salivary Cortisol (ALSPAC, NCDS, NSHD, HCS)

Each study that measured salivary cortisol, did so in a different manner; the details are as follows:

In ALSPAC, participants aged 15.5 yrs were given a cortisol sampling kit during their clinic visit. Each pack contained sampling instructions, 12 Salivette collection devices (Sarstedt, UK), a sampling collection diary sheet and a pre-paid envelope to return samples to the laboratory; participants gave four samples per day over 3 consecutives school days, and recorded sampling time. The samples were collected immediately on waking (T1), at 30 min after waking (T2), after school (T3) and before bed (T4). Participants were asked not to eat or brush teeth for at least 30 min prior to collection.[82]

In NCDS, participants received a Home Saliva Collection Kit and were asked to collect two saliva samples on the next convenient day after clinical examination, the first 45min after awaking before breakfast (time 1 or T1) and the second 3h later on the same day before lunch (time 2 or T2). They were instructed to collect saliva by chewing on a swab until it was soaked and to record the exact time of sample collection. Participants were asked to refrain from brushing or flossing their teeth and eating or drinking 15min before saliva collection and to store the sample at room temperature until posting to the laboratory[83].

In NSHD, participants were shown how to collect saliva at home using salivettes. Participants were instructed to avoid brushing or flossing their teeth, or eating, drinking or (if applicable) smoking for 30 min before taking each sample. They were asked to place a Salivette saliva swab in their mouth until it was soaked, record the date and time of collection, and store the sample in the refrigerator (but not in the freezer compartment) until posted to the laboratory in a pre-paid and protected container. Samples were taken at 9-9.30 pm on the evening before the clinic or home visit and at usual waking time and waking +30 min the following day. An additional sample was taken at the clinic or home visit around mid-morning[84],[85].

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[82] Carnegie R. et al. Cortisol awakening response and subsequent depression: prospective longitudinal study. Br J Psychiatry. 2014;204(2):137-143

[83] Geoffroy M-C. et al. Prospective Association of Morning Salivary Cortisol with Depressive Symptoms in Mid-Life: A Life-Course Study. PLoS One. 2013; 8(11): e77603.

[84] Gardner M.P. et al. Dysregulation of the hypothalamic pituitary adrenal (HPA) axis and physical performance at older ages: An individual participant meta-analysis. Psychoneuroendocrinology. 2013 Jan; 38(1): 40–49.

[85] Gaysina D. et al. Cortisol and cognitive function in midlife: The role of childhood cognition andgdfgds educational attainment Psychoneuroendocrinology. 2014 Sep; 47(100): 189–198.

In HCS, participants were sent five Salivettes (Sarstedt, UK) with which to collect saliva. They were asked to fast from midnight and then to collect a saliva sample at 0, 15, 30, 45, and 60 min after waking the following morning[86]. They remained fasting during this period and were asked to refrain from brushing their teeth to prevent contamination of the saliva samples with blood. They brought the samples with them to the clinic.

Blood Cortisol (ALSPAC, HCS)

In ALSPAC (BBS at age 8 yrs and TF3 at 15.5yrs), children attended the research clinic in the morning (8:00-11:00 hrs) after fasting from at least midnight the previous day and a venous blood sample was collected.

In HCS, blood samples from males only were obtained in a morning clinic after a 12-hour overnight fast.

Are there clinical cut points?

There are no clinical cut points.

What should be considered in analyses?

There are a number of factors to be considered when analysing salivary cortisol data.[87] Cortisol levels vary substantially throughout the day with relatively high levels on waking, a sharp rise in cortisol levels reaching a peak at approximately 30-45min after waking – the so-called 'cortisol awakening response (CAR). Cortisol values then decline throughout the day reaching a nadir at the end of the day. A delay between waking and taking sample 1 results in a reduced CAR[71], and, when possible, it is conventional to remove participants reporting delay of 10min or more from the analyses. Cortisol measurements across the day can be analysed in a number of ways, the most common measures of cortisol are the CAR, slope or decline in cortisol across the day or area under the curve.[88]

Adults have slightly higher cortisol concentrations than children. In general, cortisol concentrations in the blood are very low at bedtime and highest just after waking. Certain conditions will interfere with this pattern such as Cushing's syndrome.
Pregnancy, as well as physical and emotional stress, increases cortisol concentrations within the bloodstream. Stress can increase cortisol and levels go up significantly when sick. Cortisol concentrations in the blood may also increase as a result of hyperthyroidism or obesity. A number of drugs can also increase cortisol, particularly oral contraceptives (BNF chapter 7.3), hydrocortisone (BNF chapter 6.3.2, the synthetic form of cortisol), and spironolactone (BNF chapter 2.2.3). Hypothyroidism may decrease the concentration of cortisol in the blood. Drugs that may decrease levels include some steroid hormones (glucocorticoids, BNF chapter 6.3.2).

[86] Ward A. M. V. et al. Fetal Programming of the Hypothalamic-Pituitary-Adrenal (HPA) Axis: Low Birth Weight and Central HPA Regulation. J Clin Endocrinol Metab (2004) 89 (3): 1227-1233.
[87] Adam EK and Kumari M. Assessing salivary cortisol in large-scale, epidemiological research. Psychoneuroendocrinology. 2009 Nov;34(10):1423-36.
[88] Khoury J.E. et al. Summary cortisol reactivity indicators: Interrelations and meaning. Neurobiology of Stress, 2 (2015) 34-43

Table A26. Salivary Cortisol (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY		Valid*, N	Method of Measurement	Mean (sd), Median, IQR Males	Mean (sd), Median, IQR Females
ALSPAC	15.5 yrs	**Day1**	Enzyme Immunoassay		
		T1, 916		7.1 (4.9) 6.3 4.0-9.0	8.0 (6.1) 6.8 4.6-9.8
		T2, 924		9.9 (6.7) 8.9 6.3-11.9	13.5 (8.9) 12.5 8.3-17.1
		T3, 884		2.5 (3.5) 1.8 1.1-2.7	2.7 (3.5) 1.9 1.2-3.2
		T4, 832		1.3 (2.4) 0.6 0.3-1.2	1.5 (2.7) 0.8 0.4-1.4
		Day2			
		T1, 842		7.3 (4.5) 6.5 4.5-8.9	8.5 (5.5) 7.3 5.0-10.5
		T2, 824		9.7 (5.3) 8.6 6.5-12.1	12.6 (6.4) 11.9 8.3-16.1
		T3, 807		2.7 (5.0) 1.9 1.2-2.8	2.8 (4.1) 1.9 1.3-3.2
		T4, 767		1.2 (2.3) 0.6 0.3-1.3	1.7 (3.6) 0.8 0.4-1.5
		Day3			
		T1, 783		7.3 (4.0) 6.6 4.6-9.2	8.9 (5.6) 7.5 5.2-10.4
		T2, 766		9.3 (4.7) 8.6 6.1-11.8	12.2 (6.6) 11.3 8.0-15.2
		T3, 716		2.3 (2.5) 1.6 1.0-2.6	2.6 (2.9) 1.9 1.2-3.1
		T4, 687		1.3 (2.5) 0.6 0.3-1.1	1.3 (1.9) 0.8 0.4-1.5
UKHLS		-	-	-	-
SWS		-	-	-	-
NCDS	Baseline Follow-up	T1, 6 466 T2, 6 504	Commercial chemiluminescence immunoassay kit	20.9 (11.4) 18.8 13.0-26.4 9.0 (7.6) 7.1 4.6-10.6	21.8 (11.6) 19.6 13.7-27.6 8.1 (6.2) 6.6 4.8-9.4
NSHD	63 yrs	T0, 1 801	Assayed by radioimmunoassay; high through-put cortisol assays	21.5 (11.0) 19.4 13.9-26.9	18.7 (9.2) 17.2 12.7-23.1
		T1, 1 794		26.3 (12.4) 24.8 17.9-33.1	26.0 (11.7) 25.3 17.6-32.9
		T2, 1 880		12.1 (7.3) 10.4 7.1-15.1	10.3 (7.3) 8.5 5.8-12.5
		T3, 1 794		3.5 (3.9) 2.4 1.6-3.8	3.4 (3.5) 2.4 1.6-3.8
HCS		T=0min, 119	Time-resolved fluorescent immunoassay (DELFIA system)	12.9 (7.6) 10.7 7.6-16.7	-
		T=15min, 122		17.4 (8.3) 15.8 11.8-21.9	-
		T=30min, 121		19.9 (7.7) 19.9 14.5-24.5	-
		T=45min, 122		18.8 (7.8) 18.1 13.3-24.4	-
		T=60min, 121		16.5 (7.2) 16.8 10.2-20.7	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A26. Salivary cortisol levels (nmol/L), median, throughout the day for the four CLOSER studies that measured salivary cortisol. ALSPAC datapoints are 3-day average.

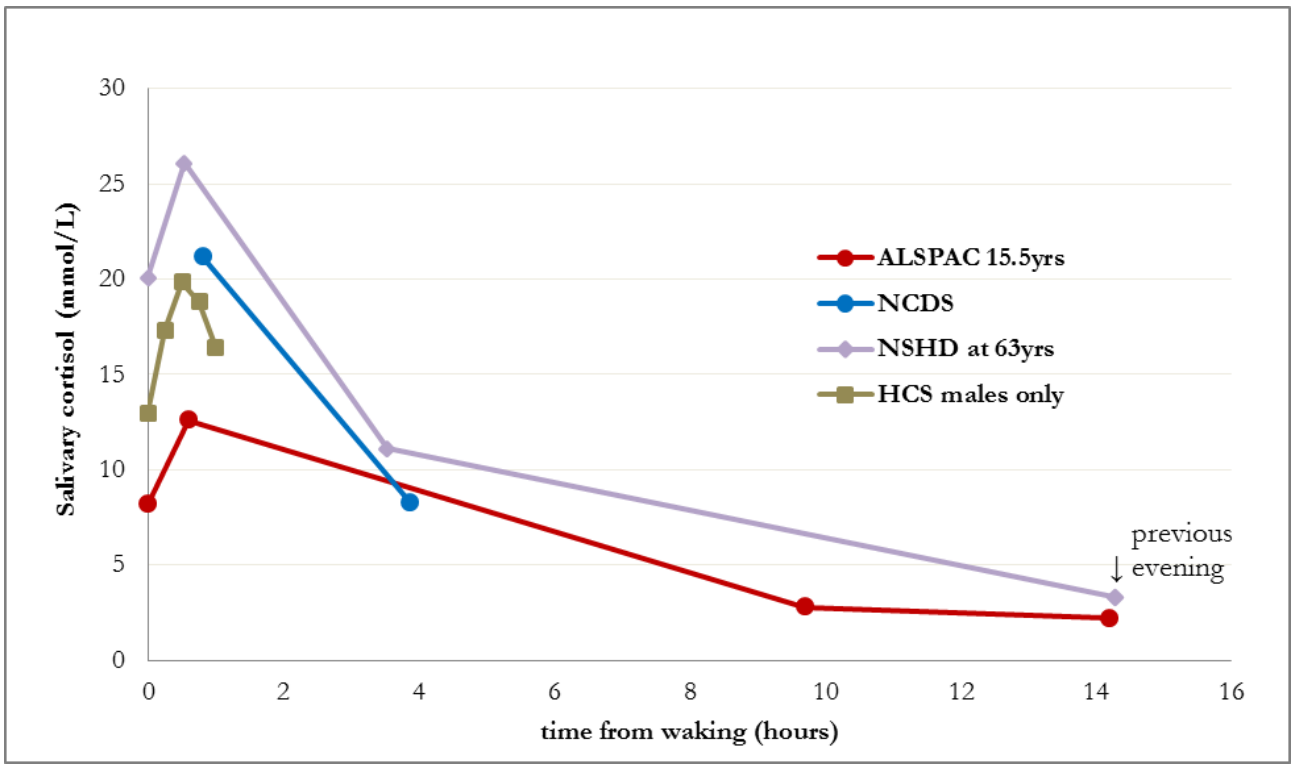
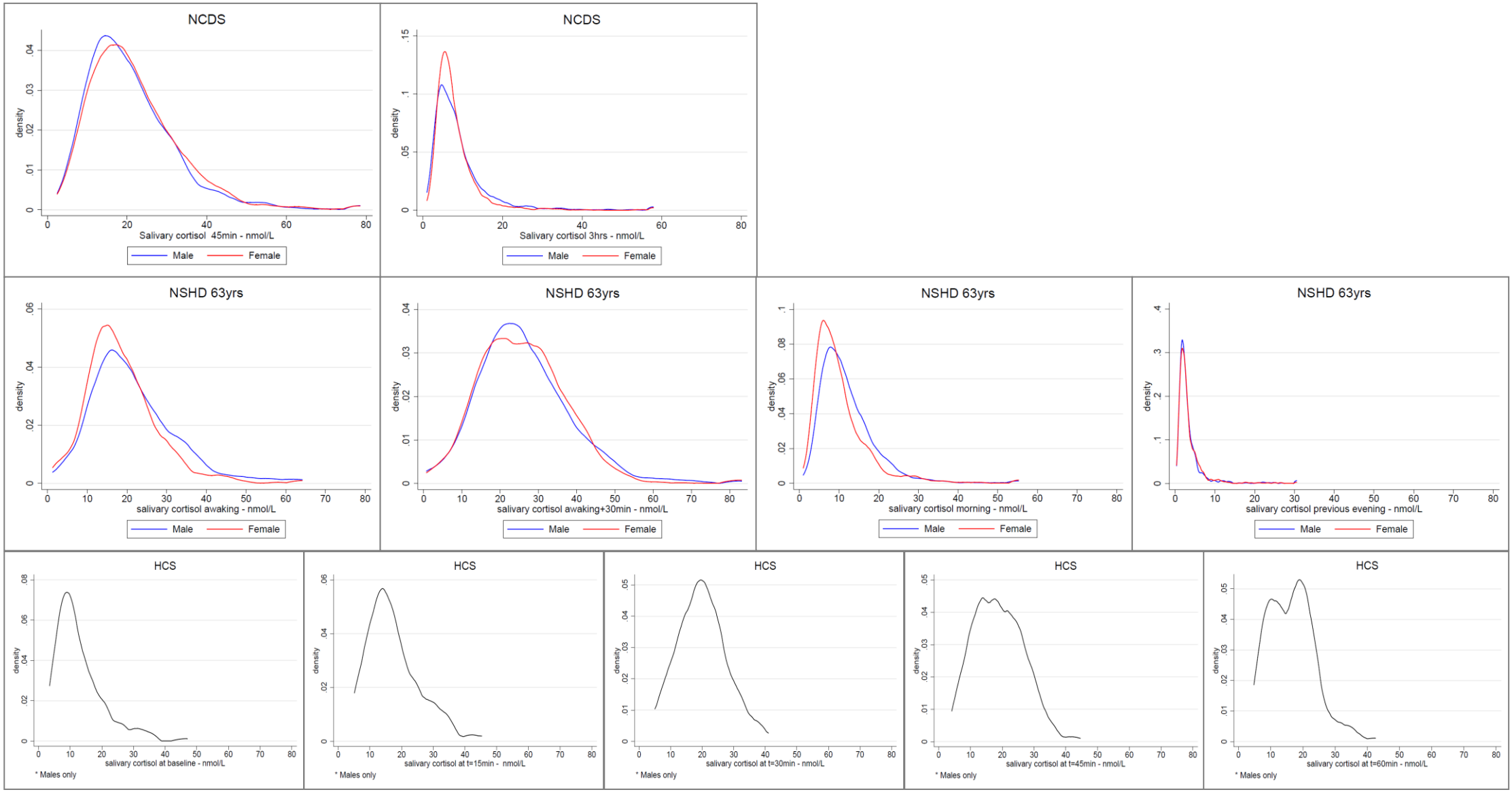
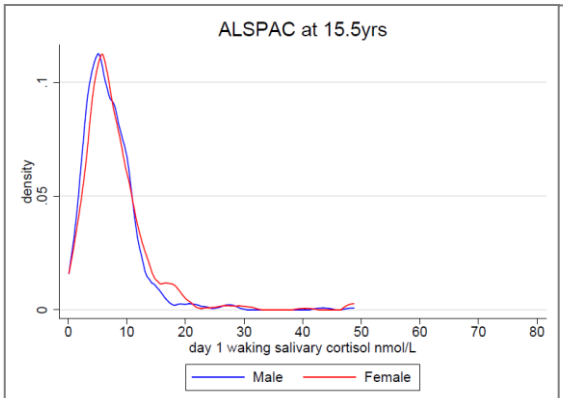
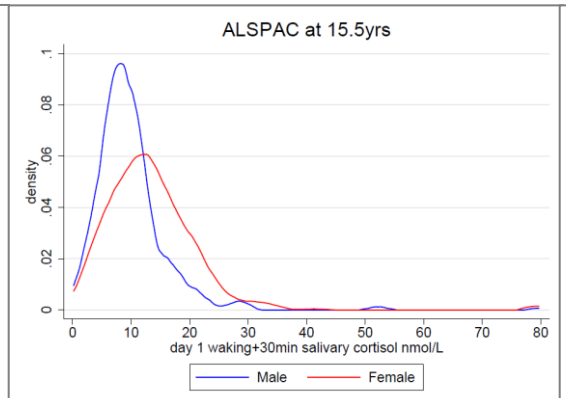
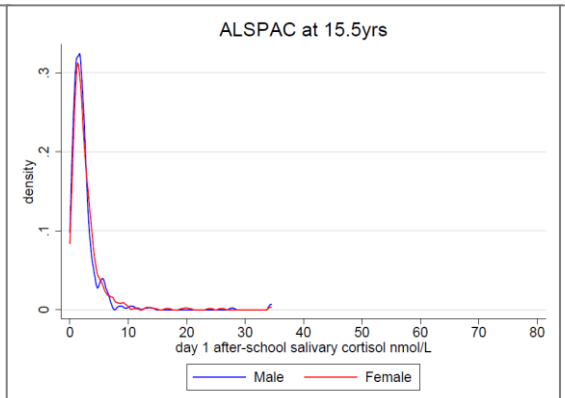


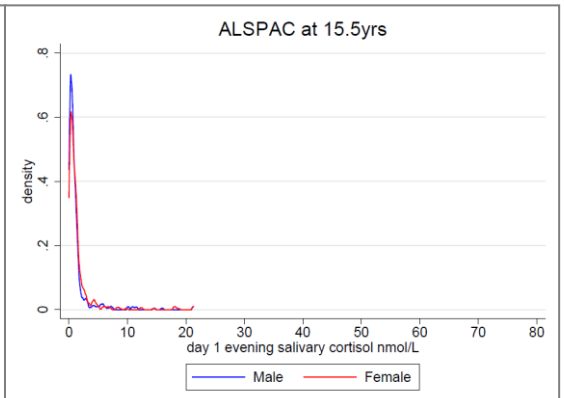
Figure B26. Distributions (Kernel density) of salivary cortisol (nmol/L) by gender for each CLOSER study that measured salivary cortisol. Distributions were winsorised at 0.5% and 99.5%.

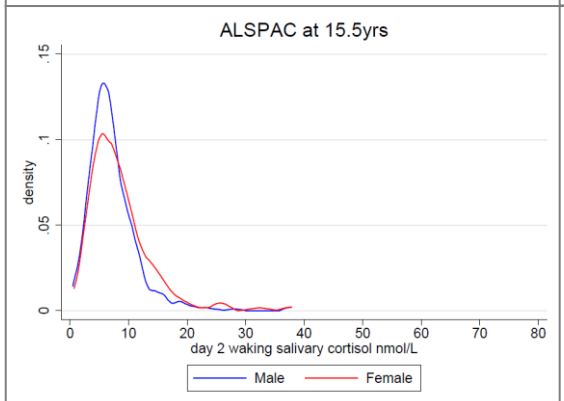


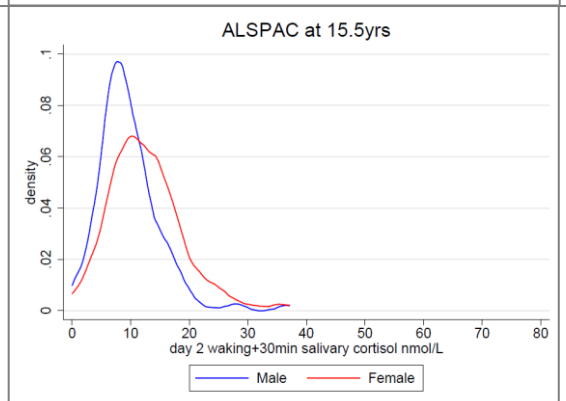

ALSPAC at 15.5yrs
density
day 1 waking salivary cortisol nmol/L
Male
Female

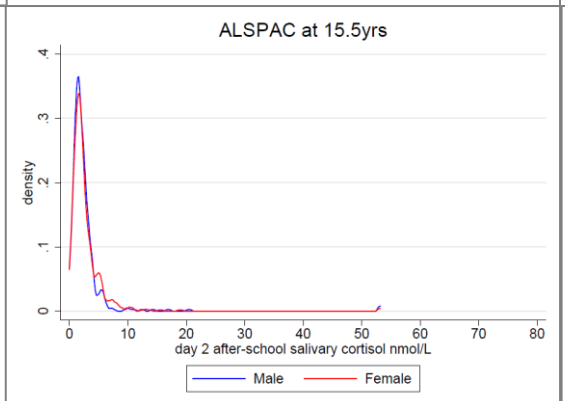

ALSPAC at 15.5yrs
density
day 1 waking+30min salivary cortisol nmol/L
Male
Female

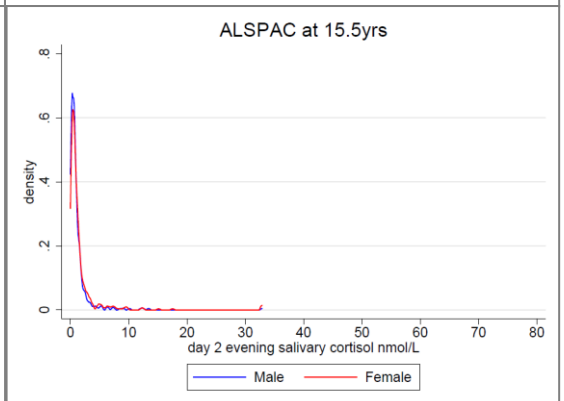

ALSPAC at 15.5yrs
density
day 1 after-school salivary cortisol nmol/L
Male
Female

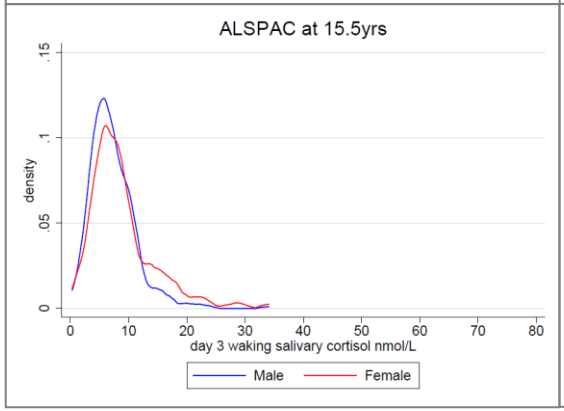

ALSPAC at 15.5yrs
density
day 1 evening salivary cortisol nmol/L
Male
Female

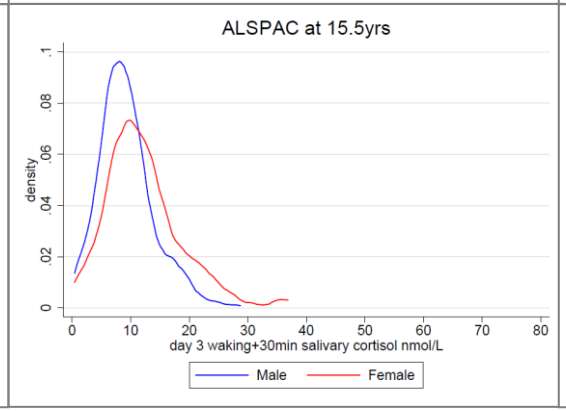

ALSPAC at 15.5yrs
density
day 2 waking salivary cortisol nmol/L
Male
Female

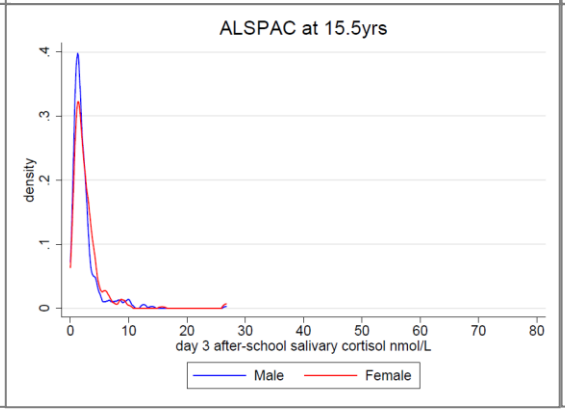

ALSPAC at 15.5yrs
density
day 2 waking+30min salivary cortisol nmol/L
Male
Female


ALSPAC at 15.5yrs
density
day 2 after-school salivary cortisol nmol/L
Male
Female


ALSPAC at 15.5yrs
density
day 2 evening salivary cortisol nmol/L
Male
Female


ALSPAC at 15.5yrs
density
day 3 waking salivary cortisol nmol/L
Male
Female


ALSPAC at 15.5yrs
density
day 3 waking+30min salivary cortisol nmol/L
Male
Female


ALSPAC at 15.5yrs
density
day 3 after-school salivary cortisol nmol/L
Male
Female

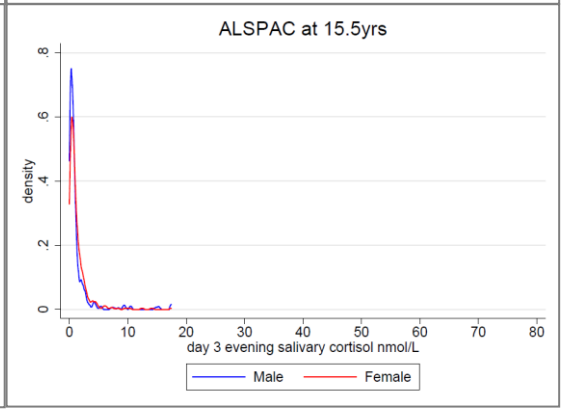

ALSPAC at 15.5yrs
density
day 3 evening salivary cortisol nmol/L
Male
Female

Table A27. Blood Cortisol (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	BBS 8 yrs 15.5 yrs	889 1 891	Immunochemiluminescence (Cortisol ELISA assay, DSL) RIA (Radioimmunoassay)	217.0 (68.0) 202.0 171.0-247.0 456.6 (138.0) 437.9 361.0-537.4	225.0 (81.0) 207.0 172.0-259.0 514.2 (194.0) 480.9 382.4-608.5
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	-		-	-	-
HCS	767		In-house RIA (Radioimmunoassay)	354.9 (108.0) 341.0 271.0-426.0	294.0 (93.0) 279.0 225.0-366.0

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A27. Crude plot of blood cortisol (nmol/L), median, by age for the CLOSER studies that measured blood cortisol**.**

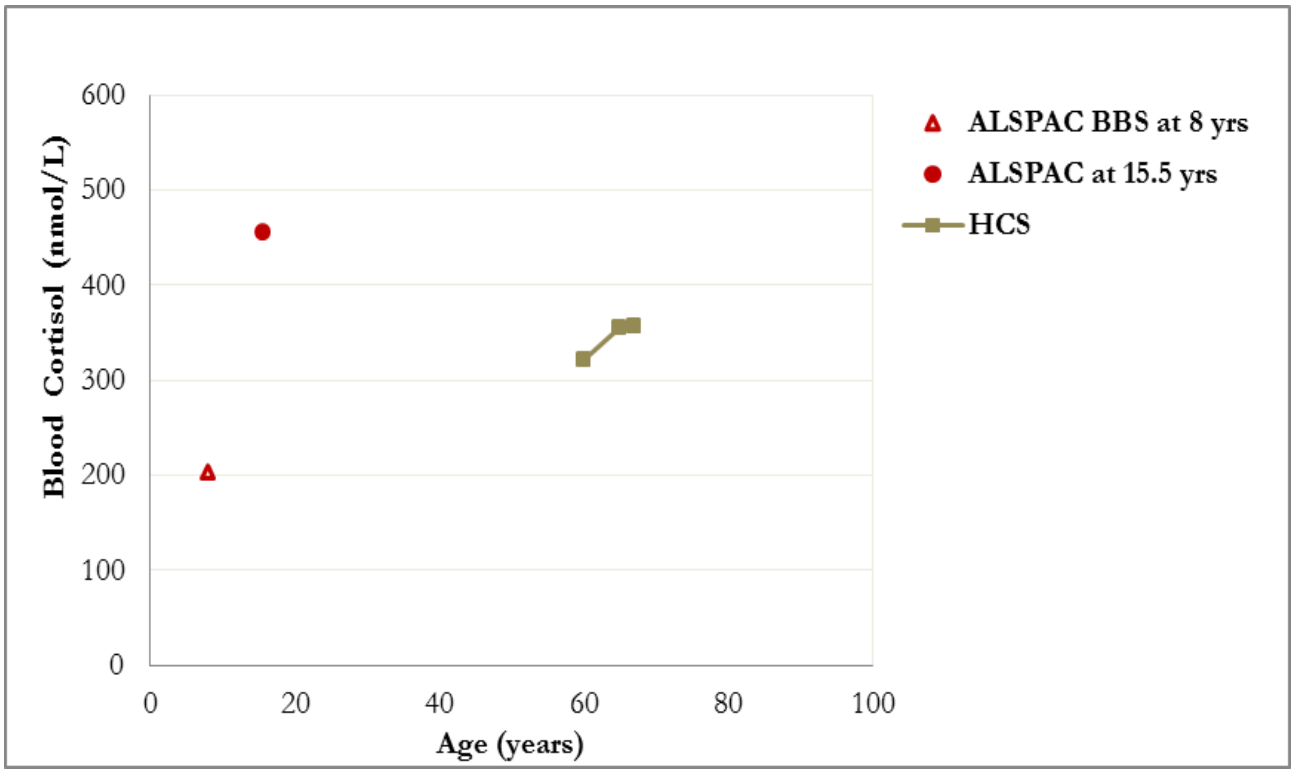
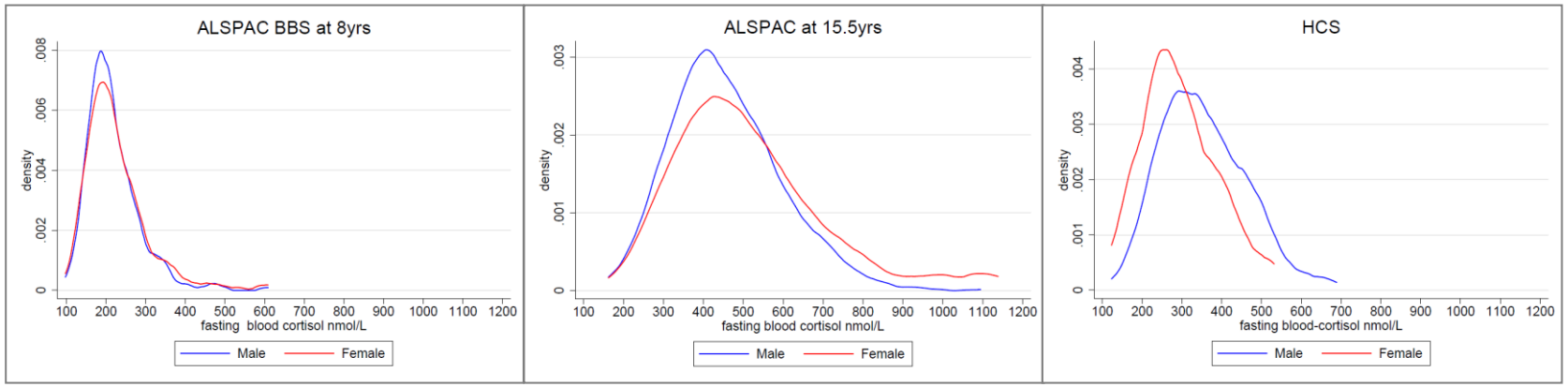


Figure B27. Distributions (Kernel density) of blood cortisol (nmol/L) by gender for each CLOSER study that measured blood cortisol. Distributions were winsorised at 0.5% and 99.5%.



Dehydroepiandrosterone Sulphate (DHEAS)

Who measured it?

DHEAS was measured by three CLOSER studies: ALSPAC, UKHLS and NSHD (see **Table 2**)

What is it?

Dehydroepiandrosterone (DHEA) and its sulphate form (DHEAs) are the most common steroid hormones in the body, and their levels decline with age[89].

What is its clinical significance?

DHEAs has been implicated in cardiovascular health; low levels are associated with CVD and all-cause mortality in older men[90], whereas higher levels are related to better health outcomes such as lower risk of metabolic syndrome[91].

How is it measured?

DHEAS is measured as described in **Table A28.**

Are there clinical cut points?

There are no established clinical cut points. The following **Table A28a** shows the expected ranges by gender and age group.

Table A28a. Expected ranges of DHEAs (μmol/L) in men and women by age group

Age range	Men	Women
15 - 19 yrs	1.9 - 13.4	1.8 - 10.0
20 - 24 yrs	5.7 - 13.4	4.0 - 11.0
25 - 34 yrs	4.3 - 12.2	2.7 - 9.2
35 - 44 yrs	2.4 - 11.6	1.7 - 9.2
45 - 54 yrs	1.2 - 9.0	1.0 - 7.0
55 - 64 yrs	1.4 - 8.0	0.5 - 5.6
65 - 74 yrs	0.9 - 6.8	0.3 - 6.7
≥ 75 yrs	0.4 - 3.3	0.3 - 4.2

What should be considered in analyses?

DHEAS concentrations are normally high in both male and female newborns. They drop sharply shortly after birth, and then rise again during puberty. DHEAS concentrations peak between the ages of 18 to 30 years and then slowly decline with age. People taking DHEA supplements will have elevated blood concentrations of DHEAS.

[89] Labrie F. et al. DHEA and the intracrine formation of androgens and estrogens in peripheral target tissues: its role during aging. Steroids, 63 (1998), 322–328

[90] Barrett-Connor E, Khaw K.T., Yen S.C.C. A prospective study of dehydroepiandrosterone sulfate, mortality and cardiovascular disease. N. Engl. J. Med., 315 (1986), 1519–1524

[91] Phillips A.C. et al. Cortisol, DHEAS, their ratio and the metabolic syndrome: evidence from the Vietnam experience study. Eur. J. Endocrinol., 162 (2010), 919–923

Table A28. DHEAs (µmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	BBS at 8 yrs	807	Immunochemiluminescence (Immulite assay)	1.5 (0.7) 1.3 1.0-1.8	1.4 (0.6) 1.2 1.0-1.7
UKHLS	12 873		Immune assay on the ROCHE E module analyser	5.7 (3.5) 5.0 2.9-7.8	3.7 (2.6) 3.1 1.8-5.0
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	53 yrs	2 002	Liquid chromatography mass spectrometry (LC-MS/MS)	4.9 (2.3) 4.5 3.1-6.1	4.2 (2.7) 4.2 4.0-4.4
	63 yrs	1 421		2.8 (1.4) 2.6 1.8-3.5	2.0 (0.9) 1.8 1.4-2.5
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A28. Crude plot of DHEAs (µmol/L), median, by age for each CLOSER study that measured DHEA-s.

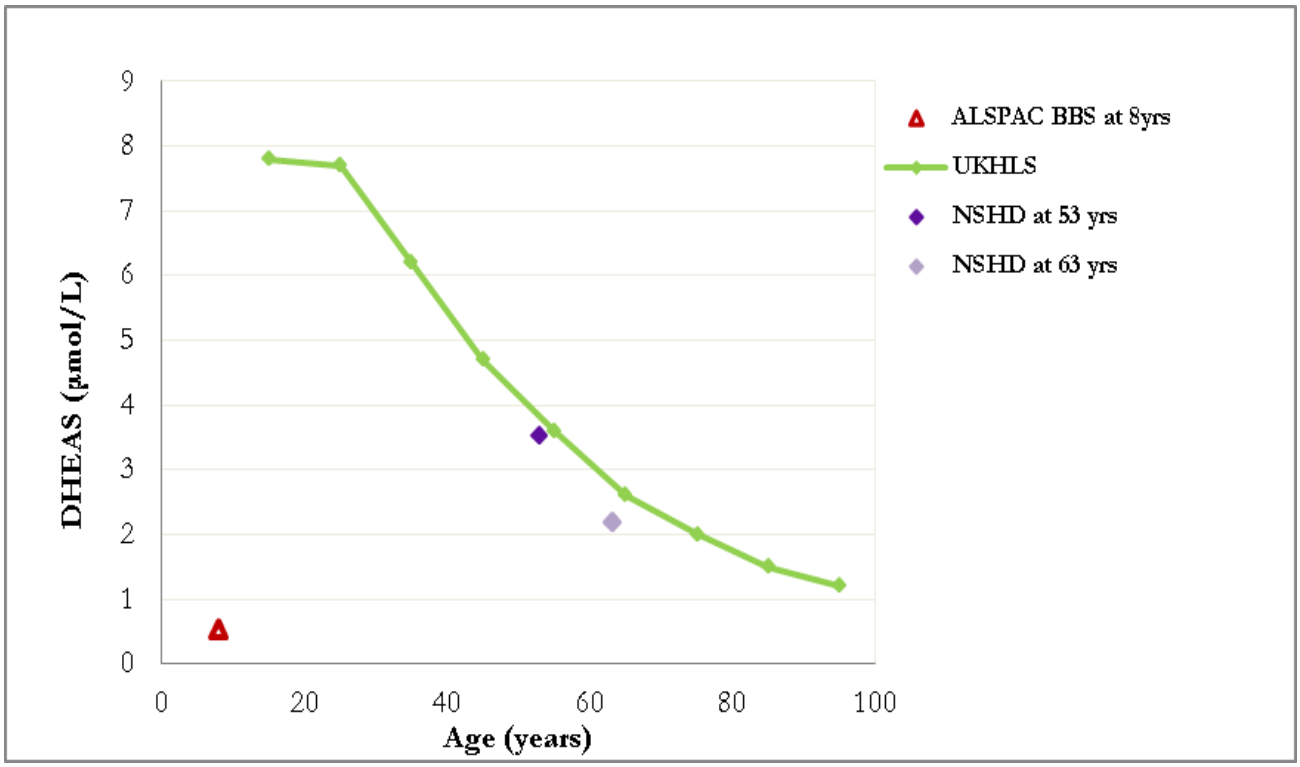
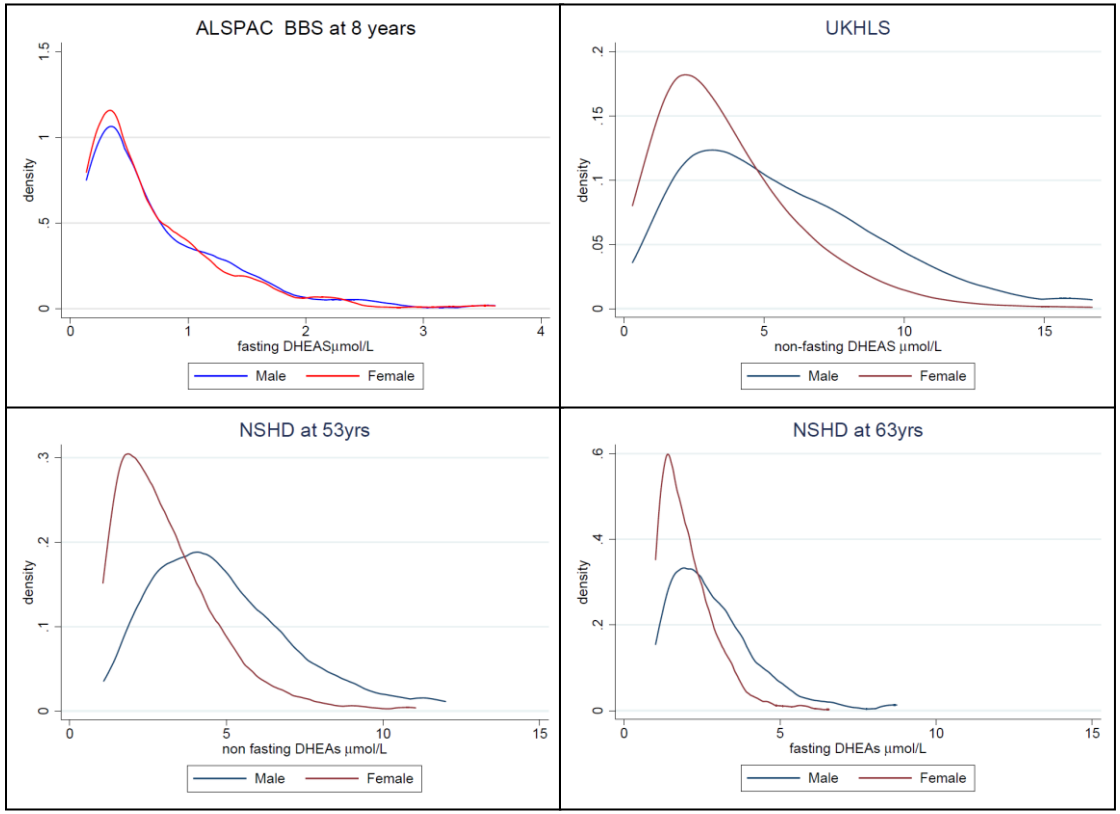


Figure B28. Distributions (Kernel density) of DHEAs (µmol/L) by gender for each CLOSER study that measured DHEAs. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



Insulin-like Growth Factor-1 (IGF-1)

IGF-1 has been measured in four CLOSER studies: ALSPAC, UKHLS, NCDS and NSHD (see **Table 2**).

What is it?

IGF-1 is a hormone, specifically an anabolic protein, which builds up organs and tissues. It plays an important role in growth and development in childhood and continues to affect adult anabolic processes.

What is its clinical significance?

Low IGF-1 levels have been shown to be associated with heart disease and high levels have been shown to be predictive of some cancers[92,93]

How is it measured?

IGF-1 is measured as described in **Table A29.** Further technical details are found elsewhere[94].

Are there clinical cut points?

There are no published clinical cut points for insulin-like growth factor-1.Laboratory methods have developed over time to measure IGF-1, which needs to be considered when comparing values across time and across studies. Normal reference values for IGF-1 vary in men and women and, because of the strong association of IGF-1 with age, these values are provided by age group in the following **Table A29a.**

Table A29a. IGF-1 reference values (nmol/L) in men and women by age

Age groups	Men	Women
17 - 18 yrs	20 - 56	35 - 73
19 - 20 yrs	21 - 85	21 - 51
21 - 25 yrs	18 - 42	12 - 44
26 - 39 yrs	15 - 37	12 - 44
40 - 54 yrs	14 - 32	12 - 44
55 - 88 yrs	11-30	12 - 44

What should be considered in analyses?

IGF-1 concentrations can be low due to a deficiency of the growth hormone (GH) or a general decrease in pituitary function (hypopituitarism) as a result of trauma, infections, and inflammation.
Decreased levels of IGF-1 also may be seen with nutritional deficiencies (including anorexia nervosa), chronic kidney or liver disease, inactive/ineffective forms of GH, and with high doses of oestrogen.
Increased concentrations of IGF-1 are normal during puberty and pregnancy but otherwise are most frequently due to pituitary tumours (usually benign).

[92] Seccareccia E., Brodt P. The role of the insulin-like growth factor-I receptor in malignancy: An update. Growth Hormone & IGF Research 22:2012;193–199
[93] Troncoso R. et al. New insights into IGF-1 signaling in the heart. Trends Endocrinol Metab. 2014; 25:128-37
[94] Holly J.M. and Hughes S.C. Measuring insulin-like growth factors: why, where and how? J Endocrinol. 1994 Feb;140(2):165-9.

Table A29. IGF-1 (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy‡	635	Radioimmunoassay (RIA)	-	25.4 (0.4) 24.7 20.3-29.6
	BBS 8 yrs	423		20.3 (0.6) 19.8 14.4-25.3	21.6 (0.6) 21.0 16.0-26.2
	7 yrs	474		17.8 (0.5) 17.4 12.7-21.6	20.2 (0.6) 19.7 15.6-24.0
	9 yrs†	49		-	-
	11+ yrs†	30		-	-
UKHLS	12 831		Electrochemiluminescent immunoassay on IDS ISYS analyser	18.8 (7.1) 18.0 14.0-22.0	18.0 (7.3) 17.0 13.0-21.0
SWS	-		-	-	-
NCDS	7 810		Chemiluminescence immunoassay	18.6 (5.4) 18.0 15.0-22.0	18.7 (5.7) 18.0 15.0-22.0
NSHD	53 yrs	2 637	Radioimmunoassay using standardized protocols	21.1 (7.0) 20.1 16.3-24.6	19.1 (6.6) 18.1 14.5-22.7
	63 yrs	1 783		18.3 (5.9) 17.9 14.4-21.9	16.8 (5.8) 15.8 12.5-20.1
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**
† Numbers are too low for statistical calculations.
‡Approximate gestation age is 10-15 weeks.

Figure A29. Crude plot of insulin growth factor-1 (nmol/L), median, by age for each CLOSER study that measured IGF-1.

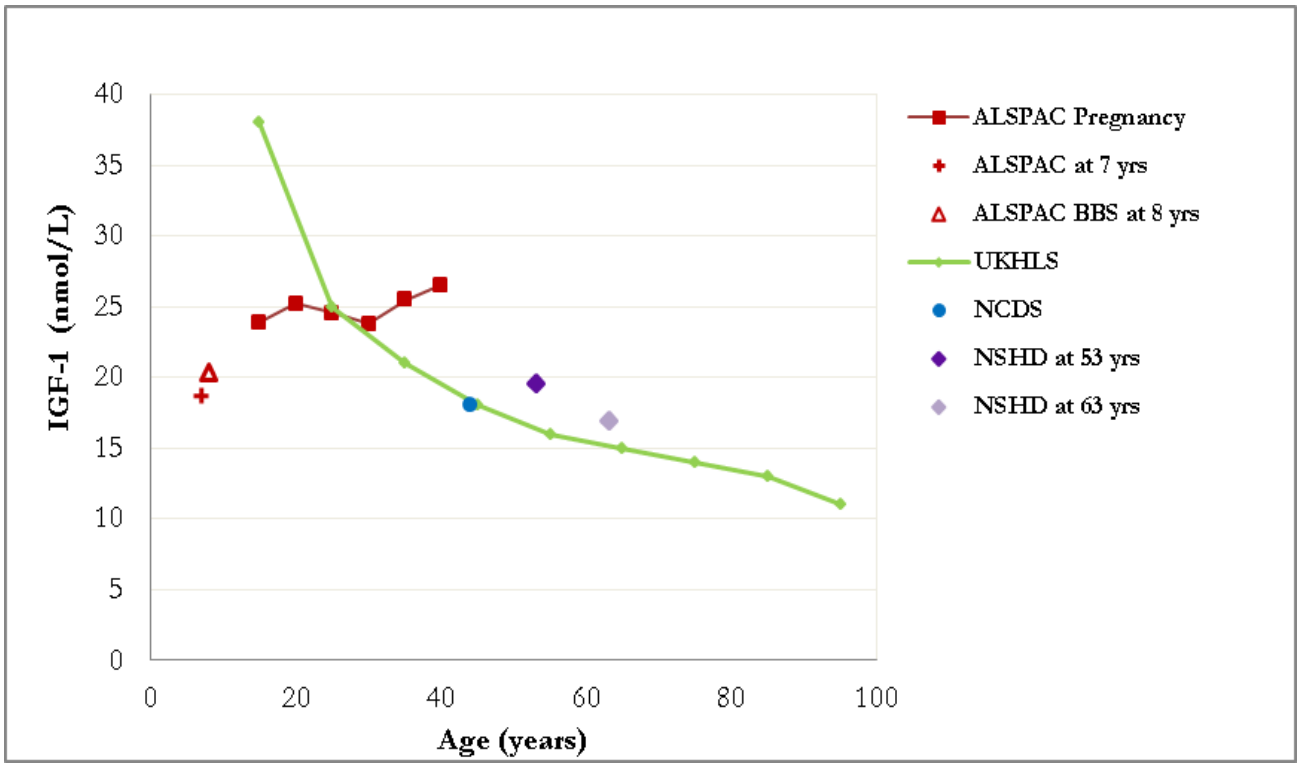
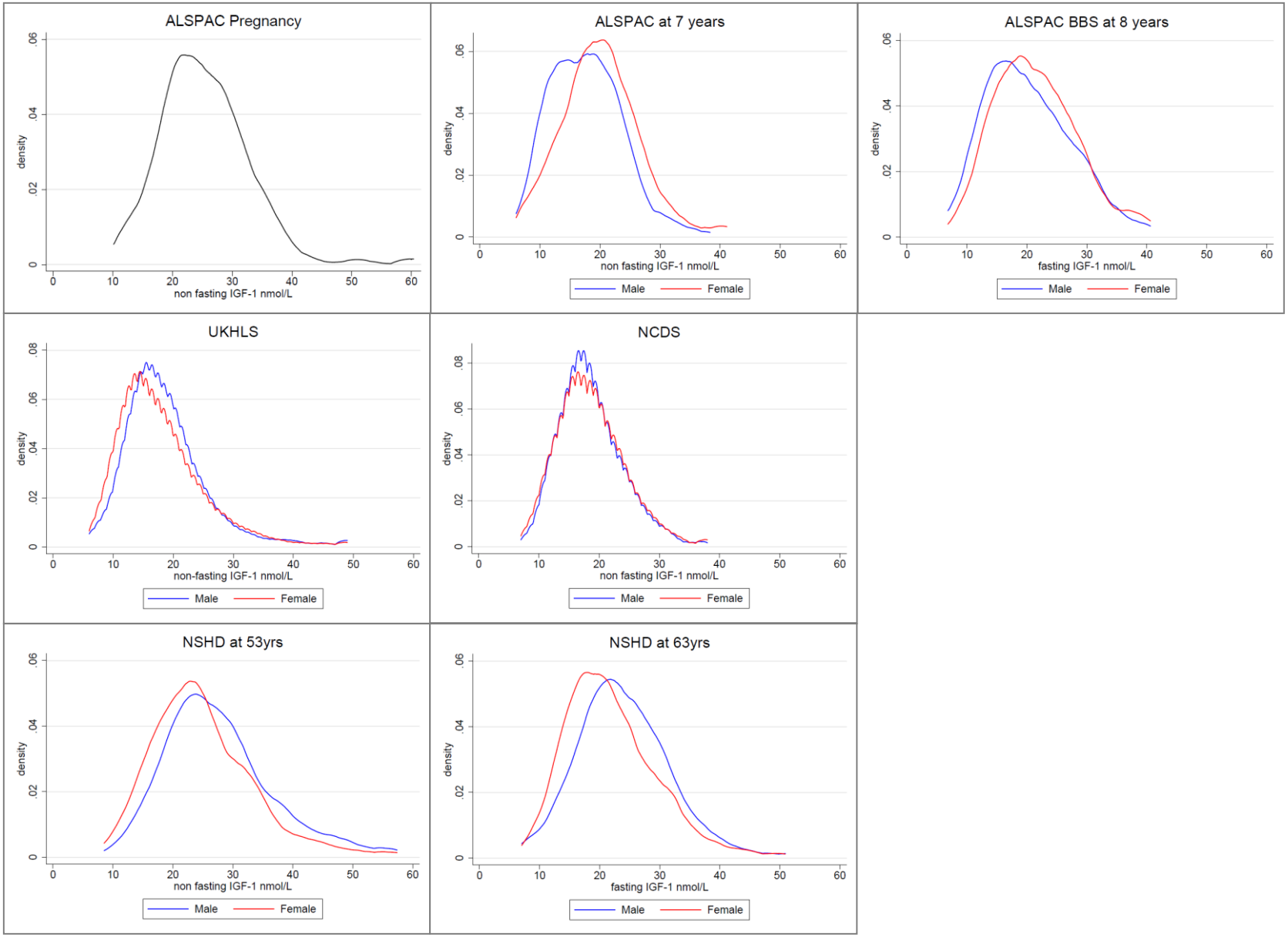


Figure B29. Distributions (Kernel density) of insulin growth factor-1 (nmol/L) by gender for each CLOSER study that measured IGF-1. Distributions were winsorised at 0.5% and 99.5%.



Insulin Growth Factor-2, IGF-2

IGF-2 has been measured in two CLOSER studies: ALSPAC and NSHD (see **Table 2**).

What is it?

IGF-2 is a hormone produced by the liver involved in the control of growth hormone levels. It is structurally similar to insulin-like growth factor-1 (IGF-1) and proinsulin. Growth hormones promote development from birth to puberty and are essential for the maintenance of metabolism, skeletal muscle, and bone tissue throughout life.

What is its clinical significance?

Altered IGF-2 levels have been observed in metabolic conditions, obesity, diabetes, polycystic ovary syndrome, liver disease and cancer.

How is it measured?

IGF-2 is measured as described in **Table A30.**

Are there clinical cut points?

There are no published clinical cut points for insulin-like growth factor-2. There is a strong association of IGF-2 with age, these values are provided by age group in the following **Table A30a.**

Table A30a. IGF-2 reference values (ng/mL) by age

Age groups	IGF-2
Pre-pubertal child	334-642
Pubertal child	245-737
Adult	288-736

What should be considered in analyses?

IGF-2 vary with age; pregnant women have altered IGF-2; individuals with certain conditions such as hypoglycemia (low blood glucose levels), hepatoma (liver disease) or Wilms tumor (kidney cancer, most common in children) may present with high levels of IGF-2 in blood samples. Certain medications, including herbal supplements, can alter the levels of IGF-2.

Table A30. Insulin Growth Factor-2, IGF-2 (ng/mL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy	589		-	637.0 (206.2) 588.3 487.7-730.1
	7 yrs	211		454.0 (104.6) 440.3 382.3-509.4	486.1 (107.6) 466.9 421.3-540.6
	9 yrs	84		683.8 (127.8) 669.7 593.2-775.0	753.6 (182.3) 737.9 608.9-889.1
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	53 yrs	2 638		745.3 (253.0) 712.4 577.0-893.4	787.9 (251.7) 763.3 606.8-942.9
	63 yrs	1 783		642.7 (295.6) 577.2 424.0-816.2	705.7 (289.2) 673.8 493.0-888.6
HCS	-		-	-	-

Figure A30. Crude plot of Insulin Growth Factor-2 (ng/mL), median, by age for each CLOSER study that measured IGF-2.

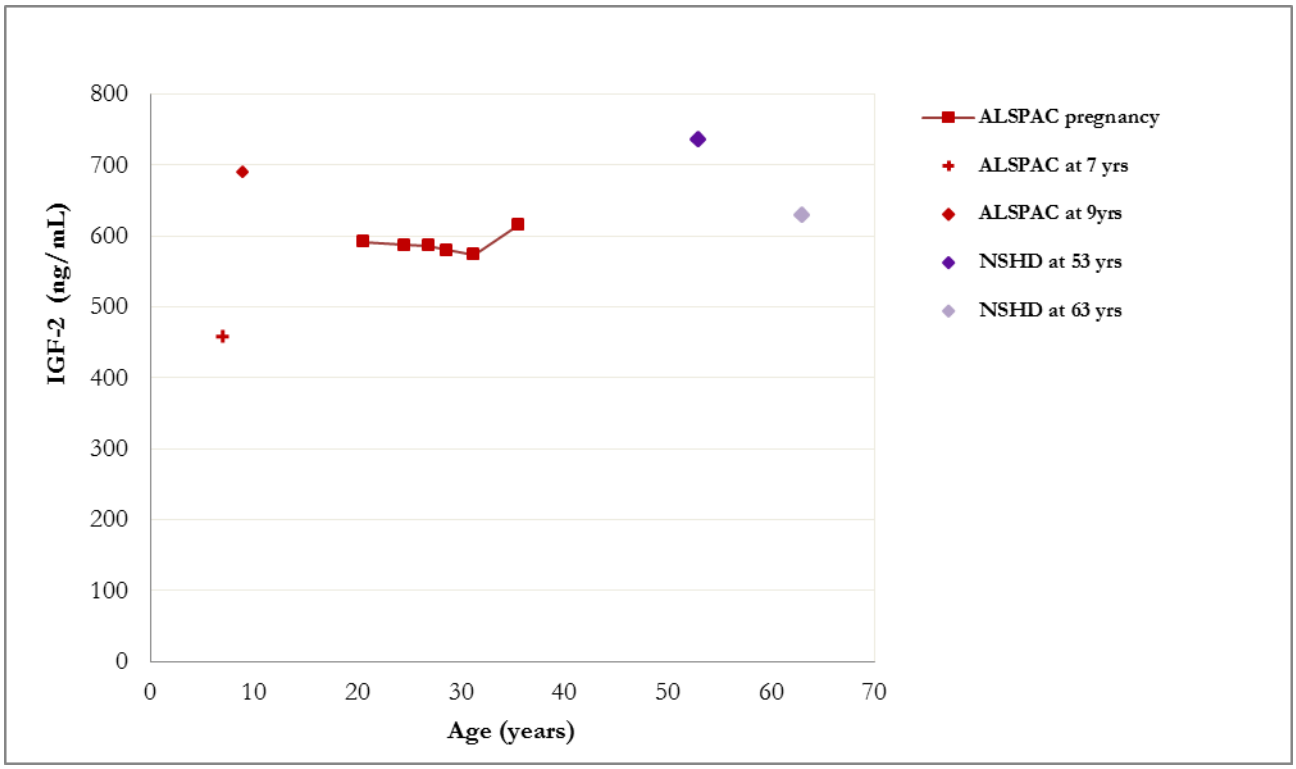
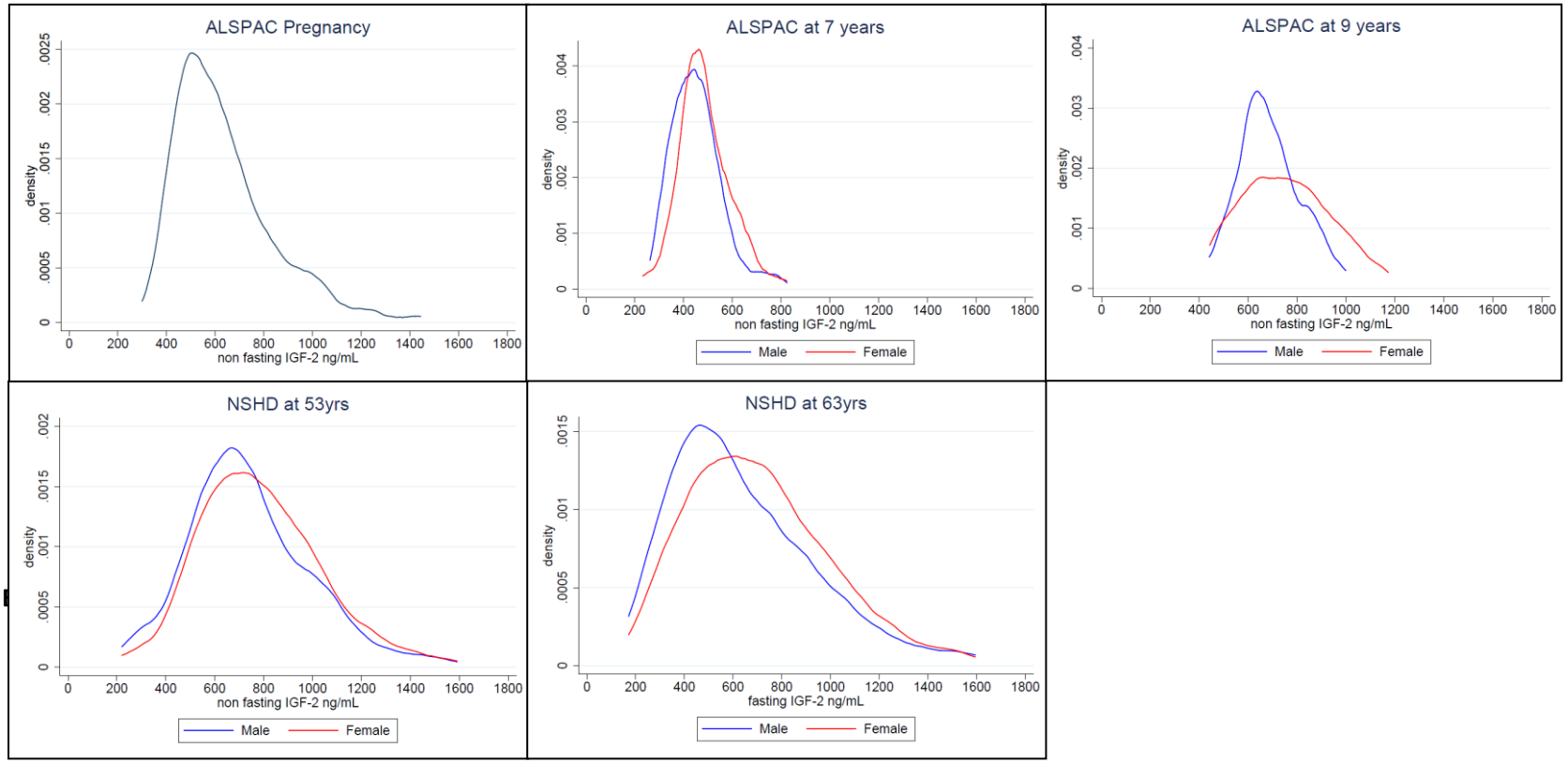


Figure B30. Distributions (Kernel density) of Insulin Growth Factor-2 (ng/mL) by gender for each CLOSER study that measured IGF-2. Distributions were winsorised at 0.5% and 99.5%.



Insulin Growth Factor Binding Protein-3, IGFBP-3

IGFBP-3 has been measured in two CLOSER studies: ALSPAC and NSHD (see **Table 2**).

What is it?

Insulin-like growth factor binding protein 3 (IGFBP-**3**) is the main carrier of somatomedin C (also called insulin-like growth factor-1, IGF-1) in the body.

How is it measured?

IGFBP-3 is measured as described in **Table 31.**

What is the clinical significance of RBC counts?

IGFBP-3 blood levels indicate whether there is a normal production of human growth hormone which in turn indicates pituitary gland disorders or abnormalities in growth hormone production.

Are there clinical cut points?

The value for the Insulin-Like Growth Factor Binding Protein-3 Blood Test is interpreted based on an individual's age, as follows:

Table A31a. IGFBP-3 reference values (ng/mL) by age

Age groups	**IGF-2**
Pre-pubertal child	500-950
Pubertal child	340-950
Adult	310-700

What should be considered in analyses?

Levels of IGFBP-3 are highest during childhood and puberty, and then they decrease during adulthood. High levels of IGFBP-3 in the blood can be caused by conditions or treatments such overproduction of growth hormone, excessive rhGH therapy (recombinant hormone Growth hormone) or chronic renal failure. Low levels can be due to growth hormone deficiency, growth hormone resistance, chronic malnutrition, liver failure or diabetes

Table A31. Insulin Growth Factor Binding Protein, IGFBP-3 (ng/mL): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy	635		-	5384.8 (1370.2) 5229.8 4337.0-6263.3
	BBS 8 yrs	423		5380.1 (1552.1) 5112.0 4368.0-6211.0	5825.7 (1762.1) 5464.0 4580.5-6660.5
	7 yrs	474		3438.7 (1002.6) 3329.3 2687.8-4064.9	3735.14 (968.9) 3607.5 3011.0-4390.0
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	53 yrs	2 638		4735.2 (1113.7) 4659.1 4040.2-5410.7	4832.8 (1118.8) 4782.5 4091.2-5513.2
	63 yrs	1 783		3204.8 (819.0) 3189.2 2679.5-3738.8	3451.1 (832.2) 3454.4 2870.0-4038.4
HCS	-		-	-	-

Figure A31. Crude plot of insulin growth factor binding protein-3 (ng/mL), median, by age for each CLOSER study that measured IGFBP-3 count.

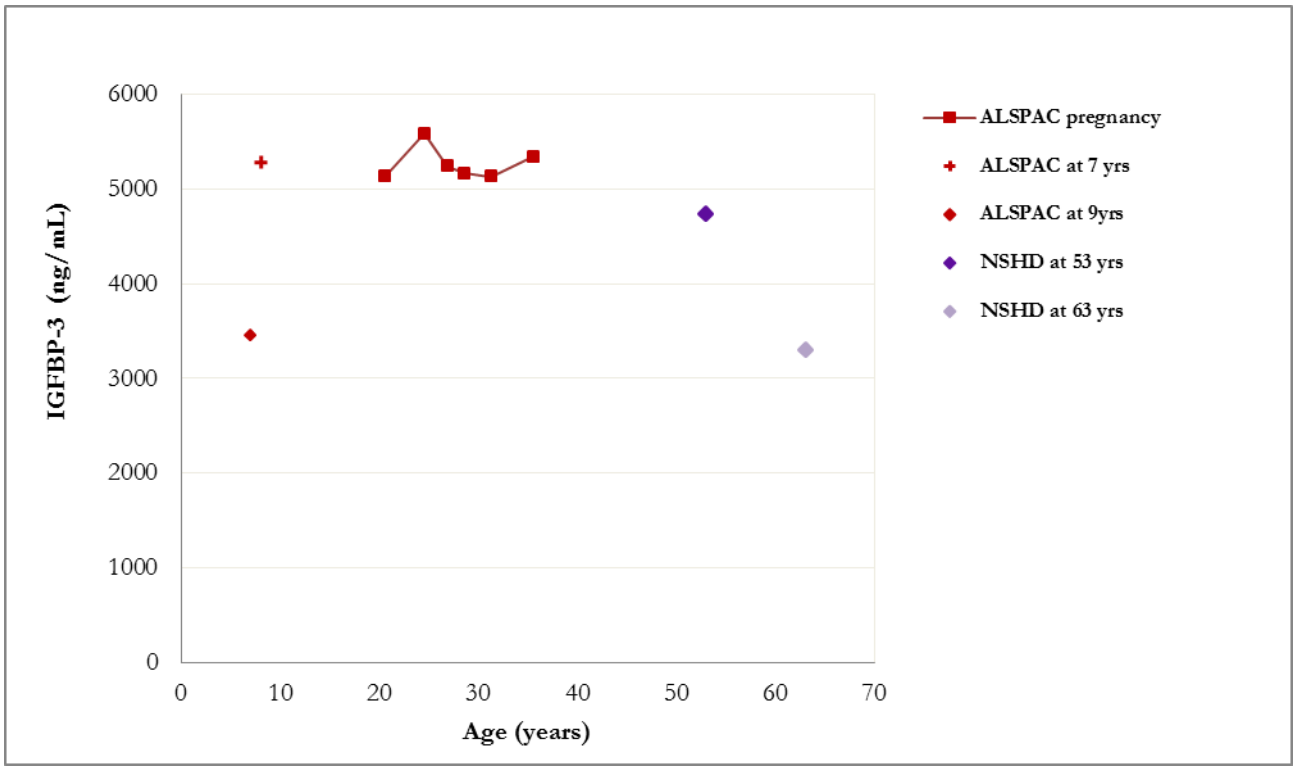
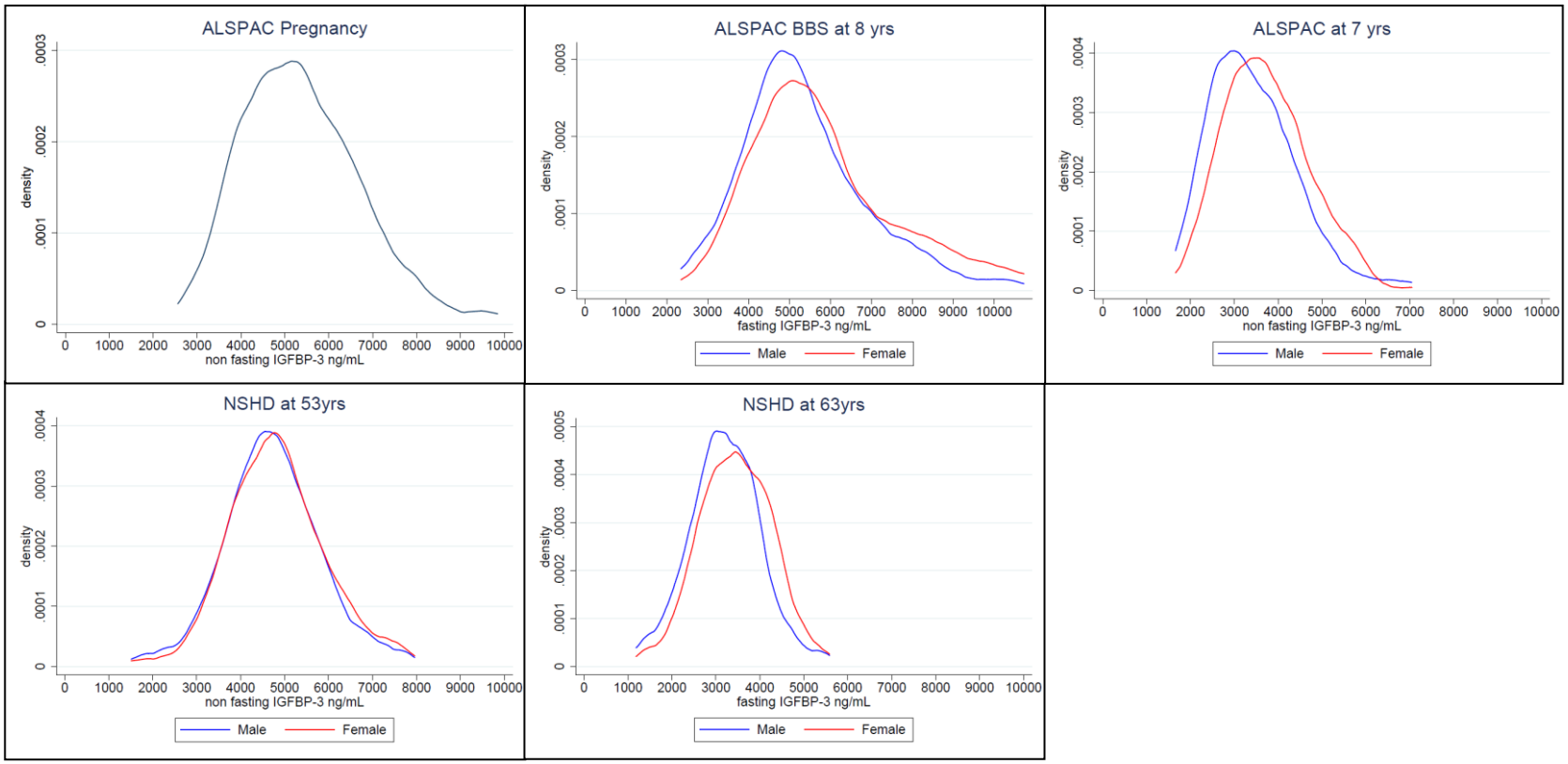


Figure B31. Distributions (Kernel density) of Insulin Growth Factor Binding Protein-3 (ng/mL) by gender for each CLOSER study that measured IGFBP-3. Distributions were winsorised at 0.5% and 99.5%.



Sex Hormone Binding Globulin (SHBG)

SHBG has been measured in two CLOSER studies: ALSPAC and NSHD (see **Table 2**).

What is it?

SHBG is a glycoprotein that binds to the two sex hormones: androgen and oestrogen. It is produced by the liver.

How is it measured?

SHBG is measured as described in **Table A32.**

What is the clinical significance of SHBG?

Measurement of SHBG is useful in the evaluation of mild disorders of androgen metabolism and enables identification of those women with hirsutism (increased hair in a woman, particularly affecting the face, thighs, chest and abdomen) that are more likely to respond to oestrogen therapy. Testosterone and SHBG ratios correlate well with both, measured and calculated values of free testosterone, and help to discriminate subjects with excessive androgen activity from normal individuals. Hyperandrogenic conditions are associated with serious health problems like insulin resistance (a precursor to diabetes), diabetes and heart disease.

Are there clinical cut points?

There are no clinical cut points for SHBG as clinicians tend to measure testosterone or other steroids for clinical assessments.

What should be considered in analyses?

The amount of SHBG in the bloodstream is affected by age and sex and by decreased or increased testosterone or oestrogen production. The aging process, particularly for women, decreases SHBG levels, which means younger women tend to have higher SHBG levels than postmenopausal women. Pregnancy increases SHBG, as SHBG is produced in the placenta tissue. Some evidence suggests that continuous use of oral contraceptives (BNF chapter 7.3) can also increase your SHBG levels. Finally, undernourishment, as seen in anorexia nervosa, and estrogen or thyroid hormone treatment can cause higher than normal SHBG levels in women. . SHBG levels are also affected by certain diseases and conditions such as liver disease, hyperthyroidism or hypothyroidism, by obesity, and by anticonvulsant drugs like phenytoin and phenobarbitone (BNF chapter 4.8).

Table A32. Sex Hormone Binding Globulin, SHBG (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy†	234	Cobas Auto Analyzer	-	241.8 (139.3) 221.5 152-301
	BBS 8 yrs	870		81.7 (31.2) 76.8 59.4-101.3	72.9 (30.9) 68.5 52.5-89.0
	15.5 yrs	1 728		-	63.9 (35.7) 57.2 39.8-79.2
UKHLS	-		-	-	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	53 yrs	1 706	Immunoassay	35.4 (15.4) 32.9 24.7-42.5	61.9 (37.6) 52.6 34.9-80.3
	63 yrs	1 783		35.7 (14.3) 33.0 25.8-42.9	50.2 (25.5) 45.0 32.8-63.6
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**
†Mean (SD) gestation age is 14 (8) weeks

Figure A32. Crude plot of sex hormone binding globulin (nmol/L), median, by age for each CLOSER study that measured SHBG.

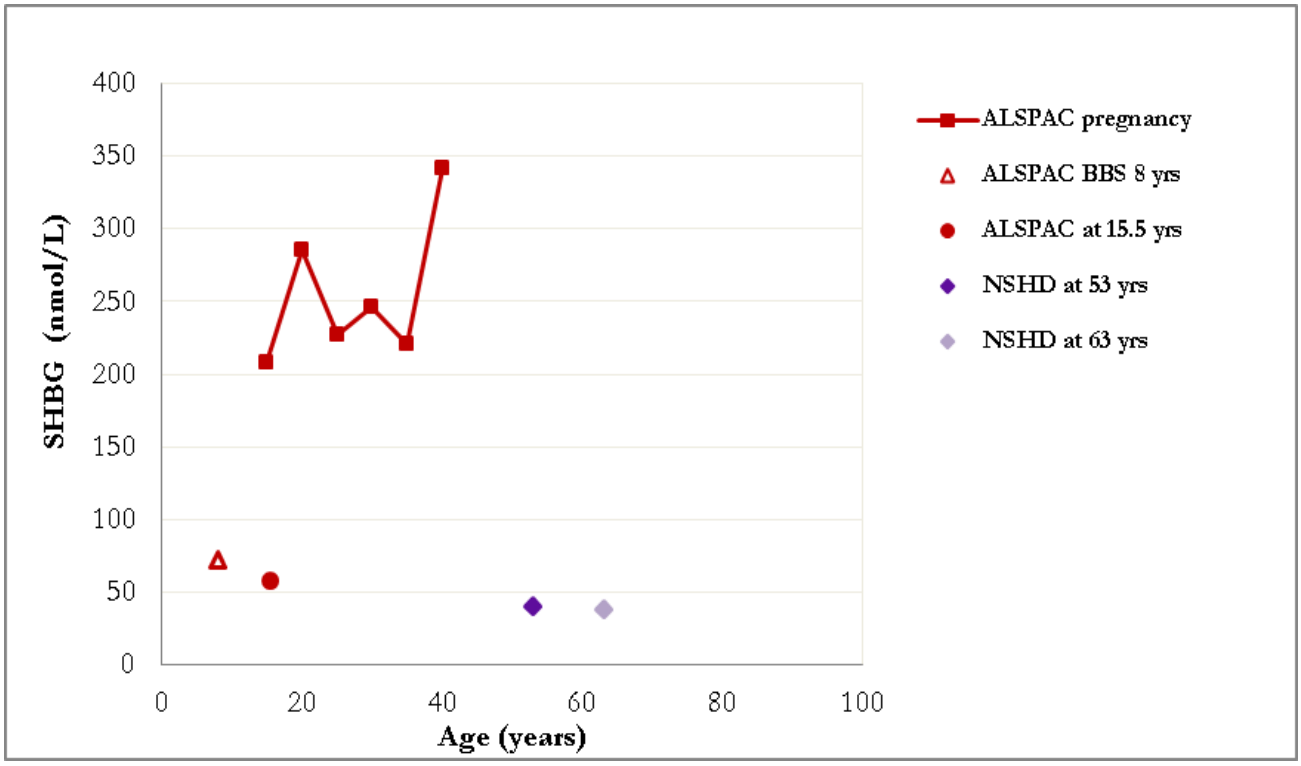
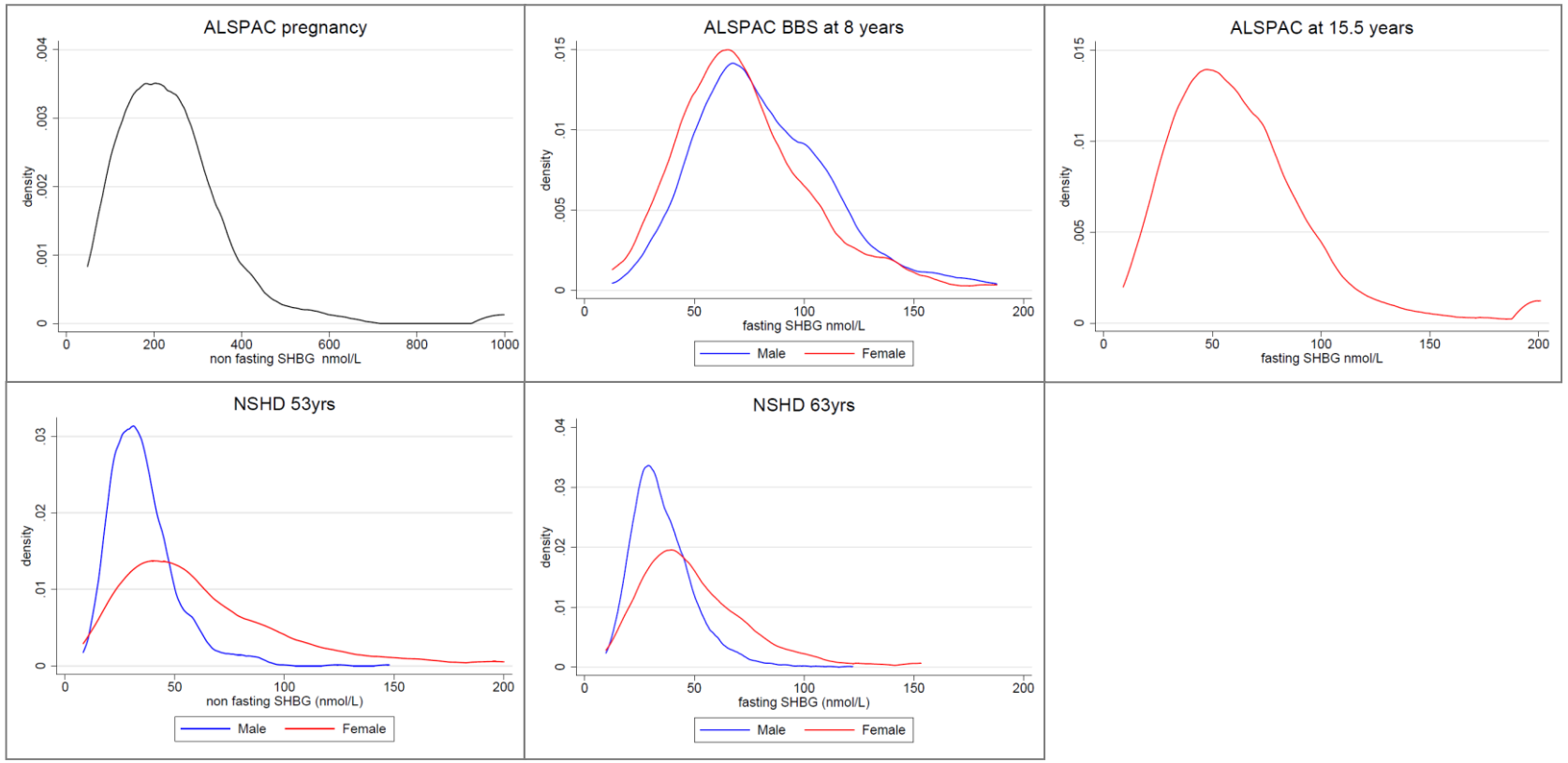


Figure B32. Distributions (Kernel density) of sex hormone binding globulin (nmol/L) by gender for each CLOSER study that measured SHBG. Distributions were winsorised at 0.5% and 9 9.5%. Note the different horizontal scales.



Testosterone

Testosterone has been measured in three CLOSER studies: ALSPAC, UKHLS and NSHD (see **Table 2**).

What is it?

Testosterone is an anabolic steroid, which builds up muscles and tissues. Testosterone is bound by carrier proteins (sex hormone binding globulin, SHBG) in the bloodstream[95,96].

What is the clinical significance of testosterone?

Testosterone is a steroid that plays a central role in the development of secondary sexual characteristics in men. It is related to libido, building muscle mass, aggression and competitive behaviours. Evidence suggests that low testosterone levels are associated with diabetes in men[97]. In women, high levels are associated with conditions such as polycystic ovarian syndrome.

How is it measured?

Testosterone is measured as described in **Table A33.** Further technical details are found elsewhere[83].

Are there clinical cut points?

Testosterone levels above or below the normal range are considered by many to be out of balance. In men, testosterone levels are wide ranging and considered within a normal range between 9-25 nmol/L and in women testosterone values are low and considered above normal when greater than 3.2 nmol/L. In UKHLS, the majority of values for women are below the lowest detection level for the analyser of 1 nmol/L, and thus are not reliable.

What should be considered in analyses?

Testosterone varies by time of day such that values in the morning are higher than those found in the afternoon or evening[98]. A total testosterone test does not distinguish between bound and free testosterone; it measures the overall quantity of testosterone. In many cases, this measurement is sufficient to discover excessive or deficient testosterone production; but, if an individual's SHBG level is not normal, then the total testosterone may be misleading. UKHLS did not measure SHBG, the chief carrier protein that binds circulating testosterone, and thus caution is required when interpreting testosterone levels.

Alcoholism and liver disease in males can decrease testosterone levels. Drugs, including androgens and steroids, can also reduce testosterone levels. Prostate cancer responds to androgens, so men with advanced prostate cancer may receive drugs that lower testosterone levels. In Females, Testosterone is produced in the ovaries and adrenal glands and increases during pregnancy.

[95] Sodergard R. et al. Calculation of free and bound fractions of testosterone and estradiol- 17 beta to human plasma proteins at body temperature. J Steroid Biochem16:801–810, 1982

[96] Vermeulen A., Verdonck L., Kaufman J.M. A critical evaluation of simple methods for the estimation of free testosterone in serum. J Clin Endocrinol. Metab. 84: 3666-3672, 1999

[97] Beatrice A.M. et al. Testosterone levels and type 2 diabetes in men: current knowledge and clinical implications. Diabetes Metab Syndr Obes. 2014 Oct 20;7:481-6.

[98] Brambilla DJ. et al. The effects of diurnal variation on clinical measurement of serum testosterone and other sex hormone levels in men. J Clin Endocrinol Metab. 94: 907-913

Table A33. Testosterone (nmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy**	234	Automated chemiluminescence system from Cheron Diagnostics	-	0.9 (0.4) 0.8 0.6-1.1
	15.5 yrs	1 782†		-	3.3 (1.4) 3.0 2.4-4.0
UKHLS	5 673‡		Electrochemiluminescent immunoassay on the Roche Modular E170 analyser	15.6 (5.7) 15.2 11.6-19.0	-
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	53 yrs	1 782	Liquid chromatography mass spectrometry (LC-MS/MS)	14.5 (5.2) 13.8 11.0-17.0	0.9 (0.5) 0.8 0.6-1.0
	63 yrs	1 655		12.6 (4.5) 12.1 9.3-15.0	0.6 (0.4) 0.6 0.4-0.7
HCS	-		-	-	-

***Valid cases include all data not coded as missing, invalid, unavailable or similar**
****Mean (SD) gestation age is 13.5 (8.0) weeks**
† Females only
‡ Males only

Figure A33. Crude plot of testosterone (nmol/L), median, by age for each CLOSER study that measured testosterone. ALSPAC at 15.5 yrs is for females only, while UKHLS data is for males only

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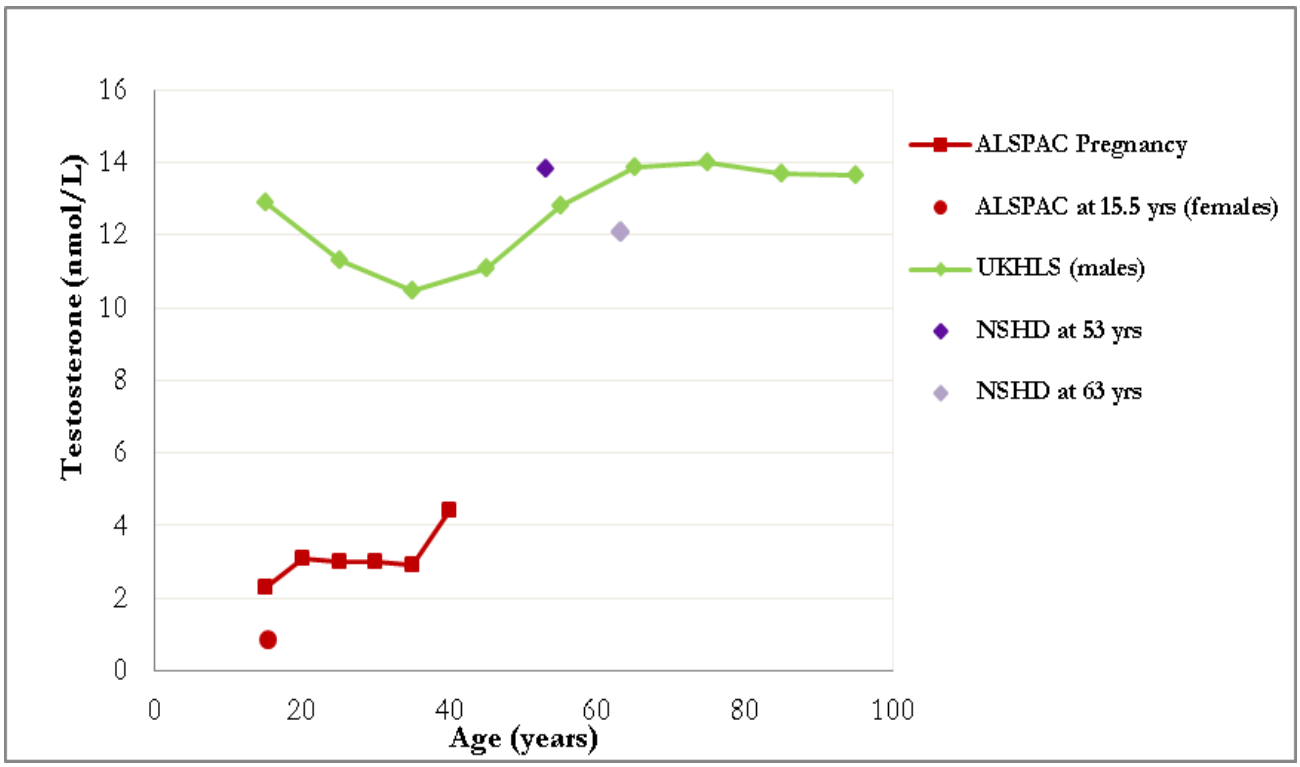
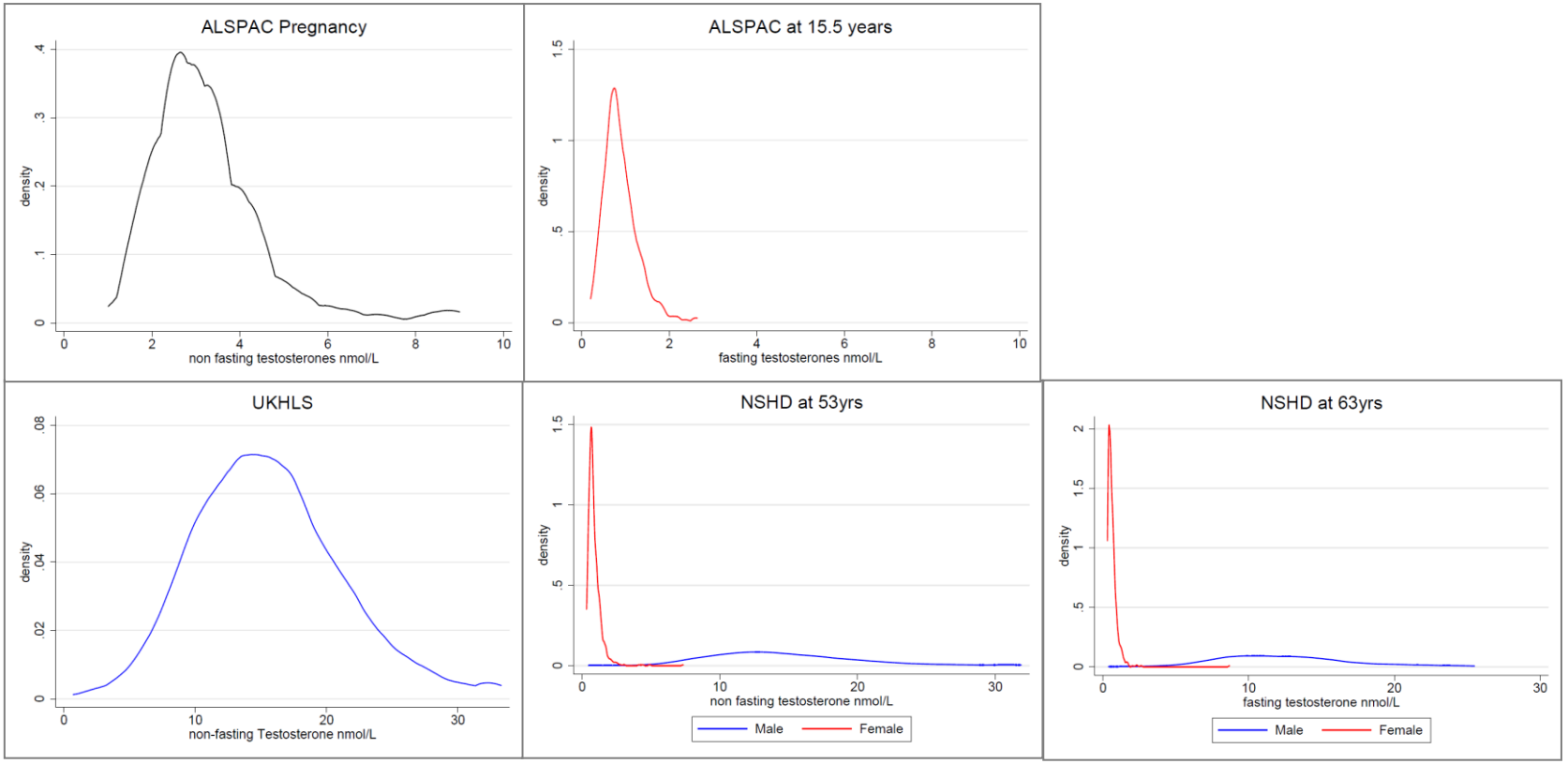


Figure B33 Distributions (Kernel density) of testosterone (nmol/L) by gender for each CLOSER study that measured testosterone. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



3.7 Kidney and Liver function Biomarkers

Calcium

Creatinine

Phosphate

Urea

Liver Function Tests (ALT/AST/ALP)

Albumin

Gamma Glutamyl Transferase (GGT)

3.7.1 Association of Kidney and Liver function markers and the social environment

Kidney Function tests

Chronic kidney disease is associated with cardiovascular disease[99] possibly through mechanisms related to hypertension. Poor kidney function and kidney disease is found to disproportionately affect those in disadvantaged social circumstances[100,101]. However, evidence is largely limited and based on international studies and requires further investigation.

Kidney function can be assessed in a number of ways including serum levels of Calcium, Creatinine and Urea. Most observational studies that examine kidney function have derived data using the serum creatinine based estimated glomerular filtration rate (eGFR).

Liver Function tests

Liver dysfunction is associated with life style behaviours, i.e., alcohol intake, but some suggest that they may also reflect inflammatory activity. In Scotland, there is a steep social gradient for chronic liver disease among men; those most deprived show a 10-fold increase in deaths compared to the most privileged groups[102].

Liver function tests include a panel of measures: Alanine transaminase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Albumin and Gamma Glutamyl Transferase (GGT).

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[99] Liu M. et al. Cardiovascular disease and its relationship with chronic kidney disease. Eur Rev Med Pharmacol Sci. 2014 Oct;18(19):2918-26.

[100] Byrne C. et al. Socioeconomic status, and the development of end-stage renal disease. Am J Kidney Dis. 1994;23(1):16–22.

[101] Al-Qaoud T.M. et al. Socioeconomic status and reduced kidney function in the Whitehall II Study: role of obesity and metabolic syndrome. Am J Kidney Dis. 2011 Sep;58(3):389-97.

[102] Equally Well: Report of the Ministerial Task Force on Health Inequalities - Volume 2 (http://www.gov.scot/Publications/2008/06/09160103/2)

Calcium

Calcium has been measured in three CLOSER studies: ALSPAC, SWS and NSHD (see **Table 2**).

What is it?

Calcium is a mineral with numerous functions, including the maintenance of bone function, nerve function and blood clotting.

What is the clinical significance of Calcium?

Measurement of calcium is not related to any specific disease diagnosis. Low levels may indicate kidney failure; abnormal levels are associated with thyroid disease, parathyroid disorder, malabsorption, cancer, or malnutrition

How is it measured?

Calcium is measured as described in **Table A34.**

Are there clinical cut points?

There are no clinical cut-points for calcium.

What should be considered in analyses?

Because about half of the calcium in the blood is bound by albumin (a protein), the albumin level in blood must be taken in to account when interpreting the calcium level. For this reason, most laboratories report a value for albumin-adjusted calcium. A frequently used formula is:

Adjusted[calcium]mmol/L= measured[calcium]mmol/L + 0.02x(40 - [measured albumin]g/L)

Such formulae however are unreliable if albumin<25 g/L. If Albumin levels are not known, a default value of 40g/L can be used.

High calcium levels can be caused by diuretic drugs (BNF chapter 2.2, drugs that encourage urination) and conditions such as hyperthyroidism, sarcoidosis and tuberculosis. Excess Vitamin D intake, kidney transplant and high protein levels can also increase calcium levels. Total calcium falls during pregnancy as a consequence of the fall in albumin.

Table A34. Calcium (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy†	7 994	Standard laboratory methods on Roche Modular analysers	-	1.5 (0.7) 1.5 0.7-2.2
	7 yrs	1 860		2.4 (0.1) 2.4 2.3-2.4	2.4 (0.1) 2.4 2.3-2.4
	9 yrs	5 082		2.4 (0.1) 2.4 2.3-2.5	2.4 (0.1) 2.4 2.4-2.5
	11^{+} yrs	1 692		2.5 (0.1) 2.4 2.4-2.5	2.4 (0.1) 2.4 2.4-2.5
UKHLS	-		-	-	-
SWS	LP‡	565		-	2.2 (0.1) 2.2 2.2-2.3
NCDS	-		-	-	-
NSHD	63 yrs	2 063	Complexation with cresolphthalein; colorimetric quantitation	2.3 (0.1) 2.3 2.2-2.3	2.3 (0.1) 2.3 2.2-2.4
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

† Average gestation age is 12-34 weeks (2nd trimester)

‡LP: Approximate gestation age is 34 weeks.

Figure A34. Plot of calcium (mmol/L), median, by age for the CLOSER studies that measured calcium.

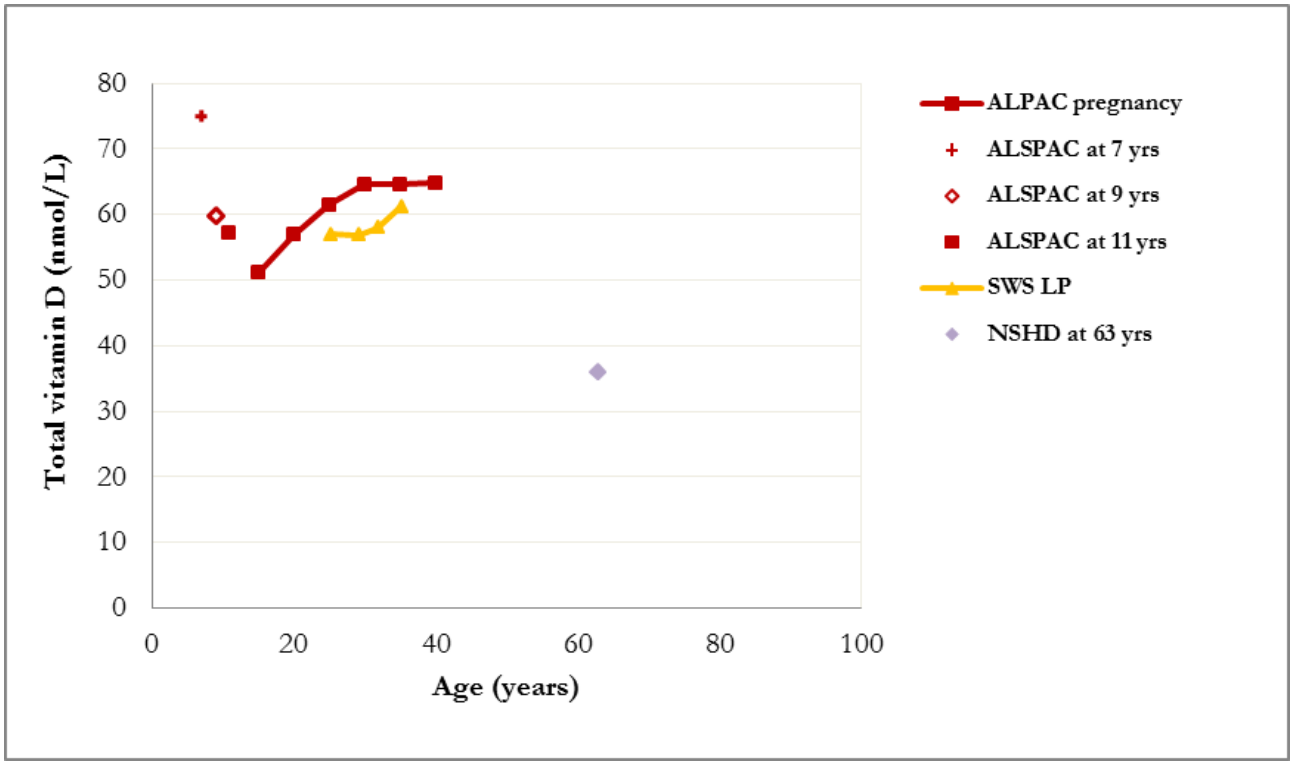
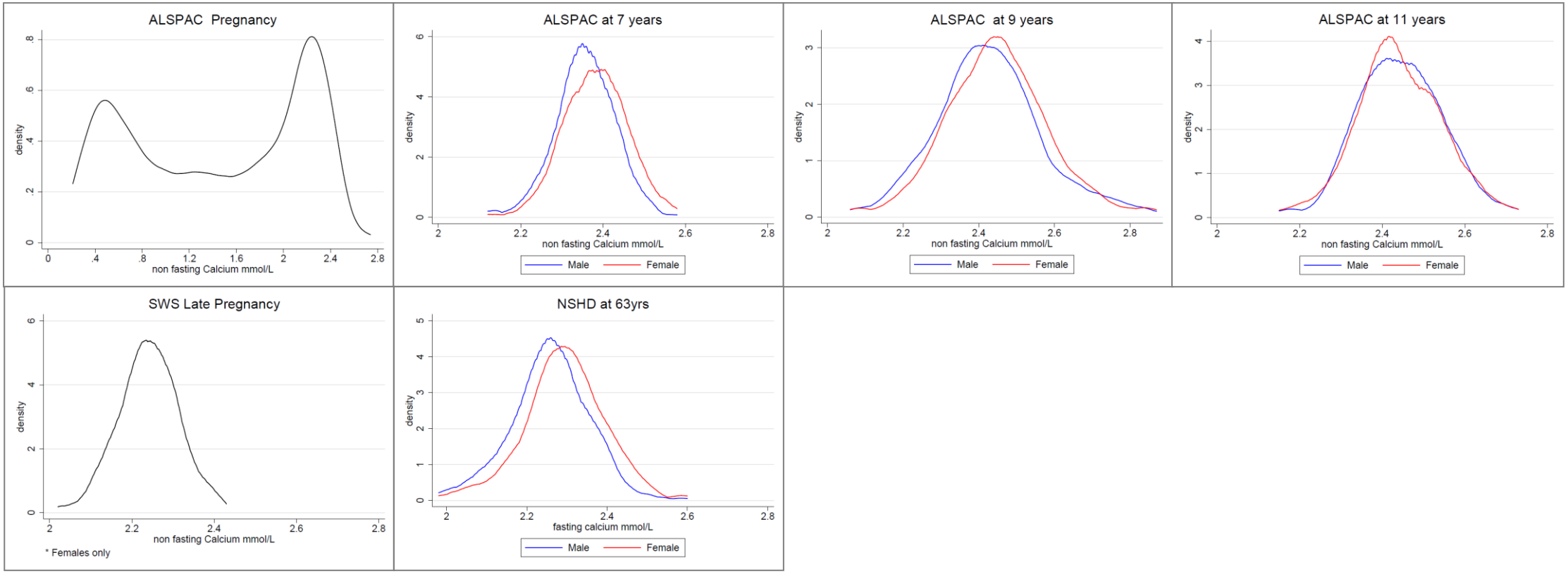


Figure B34 Distributions (Kernel density) of calcium (mmol/L) by gender for each CLOSER study that measured calcium. Distributions were winsorised at 0.5% and 99.5%. Note the different horizontal scales.



Creatinine

Urinary creatinine has been measured in three CLOSER studies: SWS, NSHD and HCS (see **Table 2**).

Blood (serum) creatinine has been measured in two CLOSER studies: UKHLS and NSHD (see **Table 2**).

What is it?

Creatinine is a chemical waste product of muscle function, which is passed through the kidneys and excreted in urine. Levels, therefore, indicate how effectively the kidneys are 'cleaning' the blood.

What is its clinical significance?

Chronic kidney disease is an increasing health problem. Creatinine is used to estimate glomerular filtration rate (eGFR), which is a standard measure of kidney function. The following **Table A35a** presents the equations to calculate eGFR based on blood (serum) creatinine; these equations have been published to identify increasing levels of kidney disease, dependent on age, gender, ethnicity and levels of serum creatinine[103].

Table A35a. Equations for estimated GFR (eGFR) based on serum (blood) creatinine (**Scr**).

Race and Sex	Serum Creatinine (Scr) μmol/L (mg/dL)	Equation (Scr in mg/dL)†
Black		
Female	≤62 (≤0.7)	$GFR = 166 \times (\mathbf{Scr}/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 166 \times (\mathbf{Scr}/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 163 \times (\mathbf{Scr}/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 163 \times (\mathbf{Scr}/0.9)^{-1.209} \times (0.993)^{Age}$
White or other		
Female	≤62 (≤0.7)	$GFR = 144 \times (\mathbf{Scr}/0.7)^{-0.329} \times (0.993)^{Age}$
	>62 (>0.7)	$GFR = 144 \times (\mathbf{Scr}/0.7)^{-1.209} \times (0.993)^{Age}$
Male	≤80 (≤0.9)	$GFR = 141 \times (\mathbf{Scr}/0.9)^{-0.411} \times (0.993)^{Age}$
	>80 (>0.9)	$GFR = 141 \times (\mathbf{Scr}/0.9)^{-1.209} \times (0.993)^{Age}$

† GFR is expressed as mL/min per 1.73 m^2 of body surface area

How is it measured?

Creatinine can be measured from urine or blood serum. Urine samples should be collected on wakening in the morning. Creatinine is measured as described in **Table A35** and **Table A36.**

Because urine creatinine is affected by delayed separation when assayed using the Jaffe reaction methods[104,105] instead of continuous flow methods, the number of suitable cases is low in NSHD data (**Table A35**).

Are there clinical cut points?

There are no clinical cut points for creatinine; however, cut points for eGFR derived from serum are:

[103] Levey A.S. et al. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009; 150:604-612.
[104] Shepherd J., Warner M.H. and Kilpatrick E.S. Stability of creatinine with delayed separation of whole blood and implications for eGFR. Ann Clin Biochem 2007; 44: 384–387
[105] Loretta F. and Jonathan B. Delay in separating blood samples affects creatinine measurement using the Roche kinetic Jaffe method. Annals of Clinical Biochemistry 2008; 45: 83–87

•stage 1 (G1) – a normal eGFR (above 90mL/min)
•stage 2 (G2) – a slightly reduced eGFR (60-89mL/min)
•stage 3a (G3a) – an eGFR of 45-59mL/min
•stage 3b (G3b) – an eGFR of 30-44mL/min
•stage 4 (G4) – an eGFR of 15-29mL/min
•stage 5 (G5) – an eGFR below 15mL/min, meaning the kidneys have lost almost all of their function

What should be considered in analyses?

Increased creatinine levels in the blood suggest diseases that affect kidney function, damage of blood vessels in the kidneys (glomerulonephritis), prostate disease, kidney stone, or other causes of urinary tract obstruction, reduced blood flow to the kidney due to shock, dehydration, congestive heart failure, atherosclerosis, or complications of diabetes. Creatinine blood levels can also increase temporarily as a result of muscle injury and are generally slightly lower during pregnancy. As creatinine levels are related to the amount of muscle the person has, low levels may be a consequence of decreased muscle mass (such as in the elderly, women and children), but may also be occasionally found in advanced liver disease.
Eating large amounts of meat may cause short-lived increases in blood creatinine levels. Taking creatinine supplements may also increase creatinine concentration. There are a few drugs that interfere with the creatinine test such as ACE inhibitors (BNF chapter 2.5.5), immunosuppressant (BNF chapters 8.2.2, 10.2.1) and chemotherapy drugs.

Table A35. Urine Creatinine (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	-	-	-	-
UKHLS	-	-	-	-
SWS	Init 704	Jaffe method on a Beckman Coulter AU5800	-	10.1 (6.5) 9.1 5.1-14.3
NCDS	-	-	-	-
NSHD	63 yrs 661	Kinetic version of the Jaffe method on a Siemens Dimension analyser	9.1 (4.8) 7.9 5.3-11.9	5.6 (3.3) 4.7 3.2-7.0
HCS	730	Vitros 950 analyser (Ortho Diagnostics)	8.9 (4.1) 8.1 5.6-11.8	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A35. Crude plot of urinary creatinine (mmol/L), median, by age for the CLOSER studies that measured urinary creatinine.

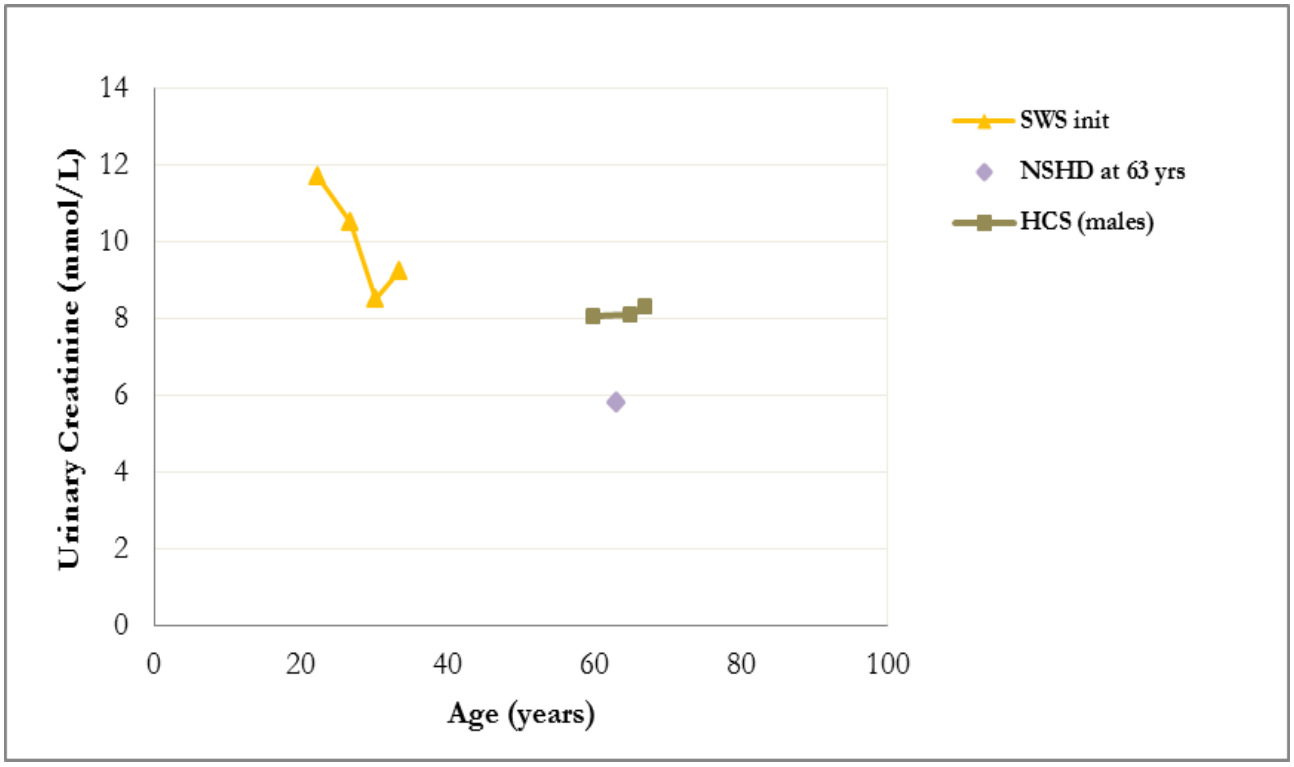


Figure B35 Distributions (Kernel density) of urinary creatinine (mmol/L) by gender for each CLOSER study that measured urinary creatinine. Distributions were winsorised at 0.5% and 99.5%.

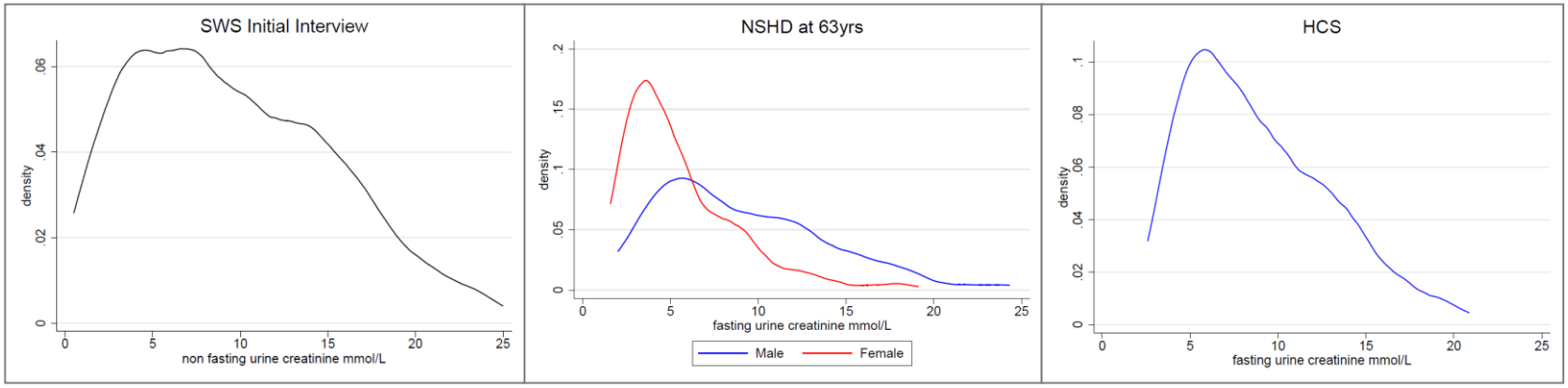


Table A36. Blood (serum) Creatinine (µmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	-	-	-	-
UKHLS	12 918	Enzymatic method on the Roche P module analyser	86.1 (16.6) 84.0 76.0-93.0	68.2 (14.1) 66.0 59.0-74.0
SWS	-	-	-	-
NCDS	-	-	-	-
NSHD	63 yrs 1 856	Kinetic version of Jaffe method on a Siemens Dimension Xpand analyser	85.9 (15.0) 84.7 74.8-94.7	67.9 (13.4) 66.9 59.1-75.0
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar**

Figure A36. Crude plot of blood creatinine (μmol/L), median, by age for the CLOSER studies that measured blood creatinine.

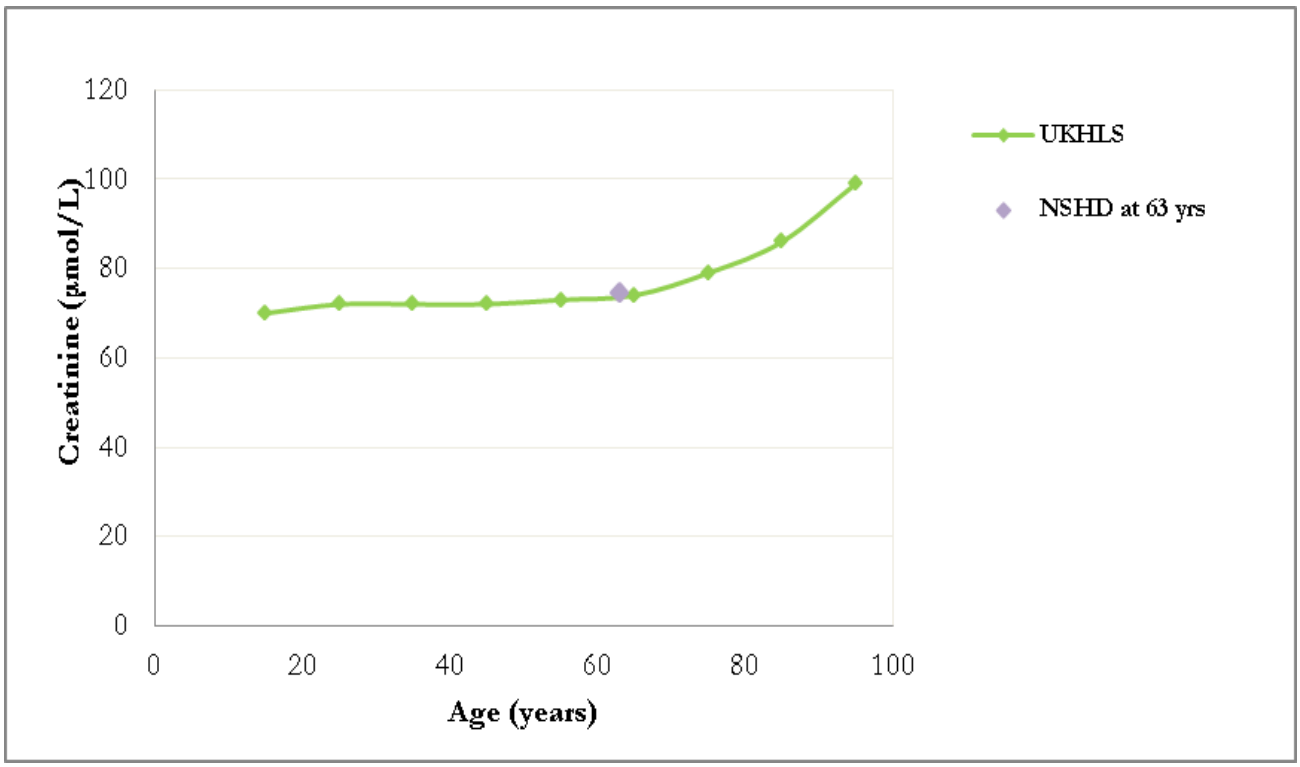
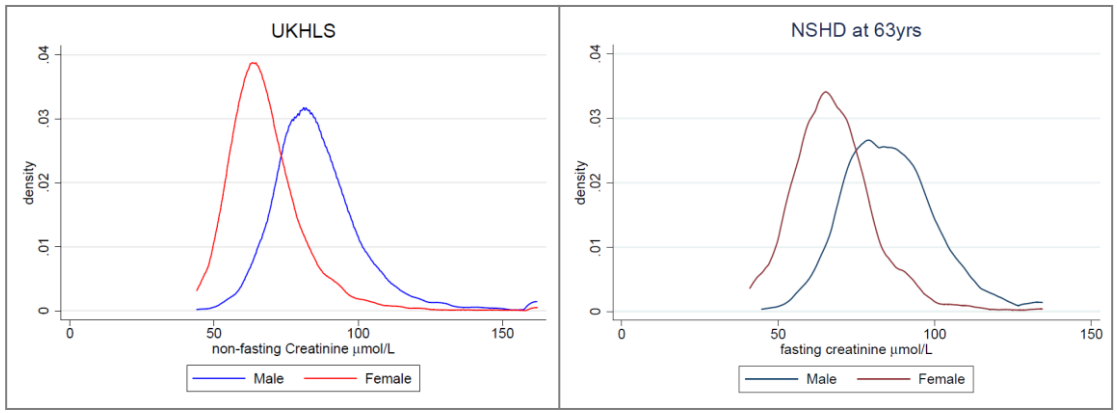


Figure B36. Distributions (Kernel density) of blood creatinine (µmol/L) by gender for each CLOSER study that measured blood creatinine. Distributions were winsorised at 0.5% and 99.5%.



Phosphate

Phosphate has been measured in two CLOSER studies: SWS and NSHD (see **Table 2**).

What is it?

Phosphates are essential for energy production, muscle and nerve function, and bone growth. Most phosphate in the body comes from dietary sources. The kidneys help control the amount of phosphate in the blood; a high level of phosphate in the blood is usually caused by a kidney dysfunction.

How is it measured?

Phosphate is measured as described in **Table A37.**

What is the clinical significance of Phosphate?

Abnormally high levels of phosphate can lead to organ damage due to calcification (calcium phosphate deposits in organs, such as the kidneys). Low phosphate levels in children can inhibit bone growth.

Are there clinical cut points?

There are no clinical cut-points.

What should be considered in analyses?

Phosphate presents high physiological variation, depending on age, gender, pregnancy, and even season (due to the seasonal variation of vitamin D which is directly involved in the regulation of phosphate concentration). Phosphate levels are normally higher in young children than in adults because their bones are actively growing. Low phosphate levels in children can inhibit bone growth. Phosphate levels may be affected by the use of enemas and laxatives containing sodium phosphate, excess Vitamin D supplements, and by intravenous glucose administration.

Table A37. **Phosphate (mmol/L)**: Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC		-	-	-	-
UKHLS		-	-	-	-
	LP‡	565		-	1.3 (0.2) 1.3 1.2-1.5
NCDS		-	-	-	-
NSHD	63 yrs	1 856	spectrophotometry	1.0 (0.1) 1.0 0.9-1.1	1.1 (0.1) 1.1 1.1-1.2
HCS		-	-	-	-

Figure A37. Crude plot of Phosphate (mmol/L), median, by age for each CLOSER study that measured phosphate.

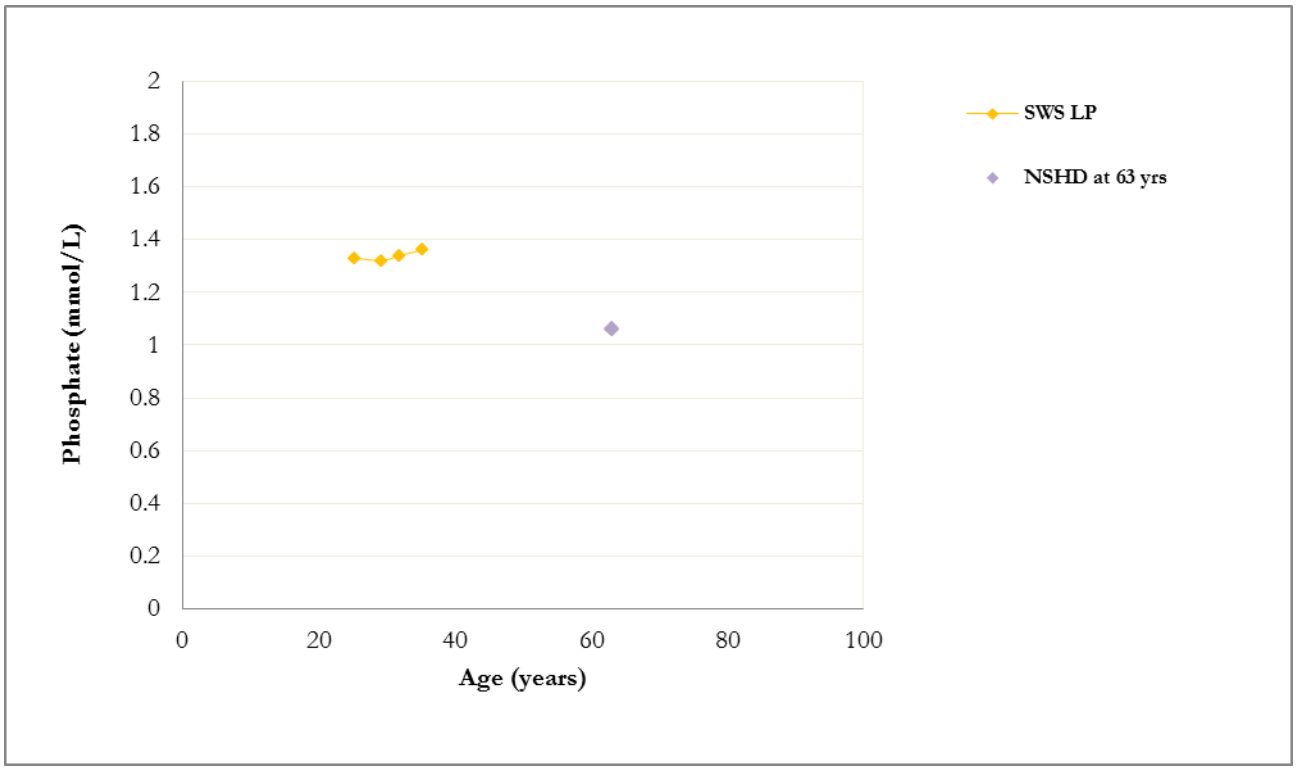
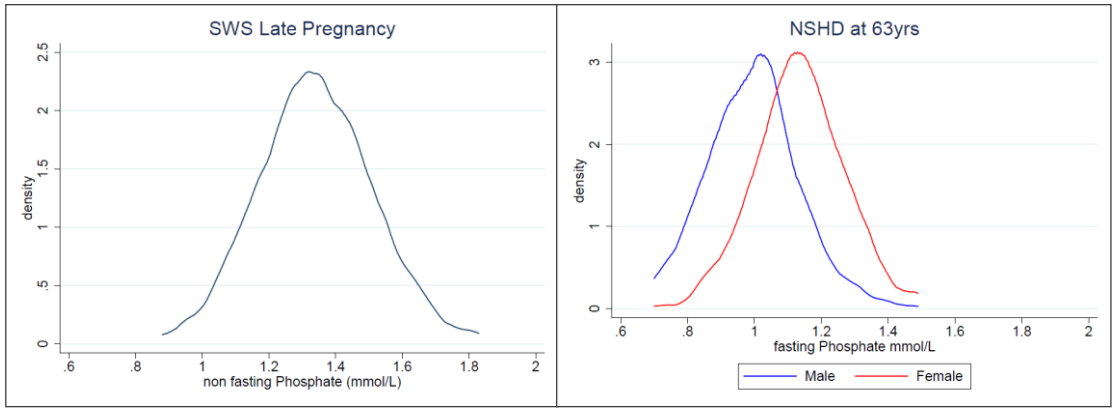


Figure B37. Distributions (Kernel density) of Phosphate (mmol/L) by gender for each CLOSER study that measured phosphate. Distributions were winsorised at 0.5% and 99.5%.



Urea

Urea (blood) has been measured in two CLOSER studies: in UKHLS and NSHD (see **Table 2**).

What is it?

Urea is a waste product of the breakdown of proteins. High levels indicate that the kidneys are not functioning effectively.

What is its clinical significance?

High urea levels indicate poor kidney function, which may be due to acute or chronic kidney disease. However, its use as a biomarker has generally been replaced by the more robust eGFR (estimate glomerular filtration rate) measure.

How is it measured?

Urea can be measure in urine and blood. Urea is measured (from blood) as described in **Table A38.**

Are there clinical cut points?

The normal range of urea is 2.5-7.8 mmol/L.

What should be considered in analyses?

Other conditions, besides kidney disease can affect urea levels such as congestive heart failure, recent heart attack, decreased blood flow to the kidneys, shock, stress, severe burns, bleeding from the gastrointestinal tract, conditions that cause obstruction of urine flow or dehydration. Low urea levels can be seen in severe liver disease or malnutrition but are not used to diagnose or monitor these conditions. Low urea levels are also seen in normal pregnancy. A protein rich diet increases urea levels.

Table A38. Urea (mmol/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	-	-	-	-
UKHLS	12 923	Kinetic UV assay on a Roche P module analyser	6.5 (1.7) 6.3 5.4-7.4	5.9 (1.6) 5.7 4.8-6.8
SWS	-	-	-	-
NCDS	-	-	-	-
NSHD	63 yrs 2 066	Kinetic enzymatic measurement using urease	5.8 (1.4) 5.7 4.8-6.7	5.6 (1.4) 5.4 4.6-6.3
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A38. Crude plot of urea (mmol/L), median, by age for the CLOSER studies that measured urea.

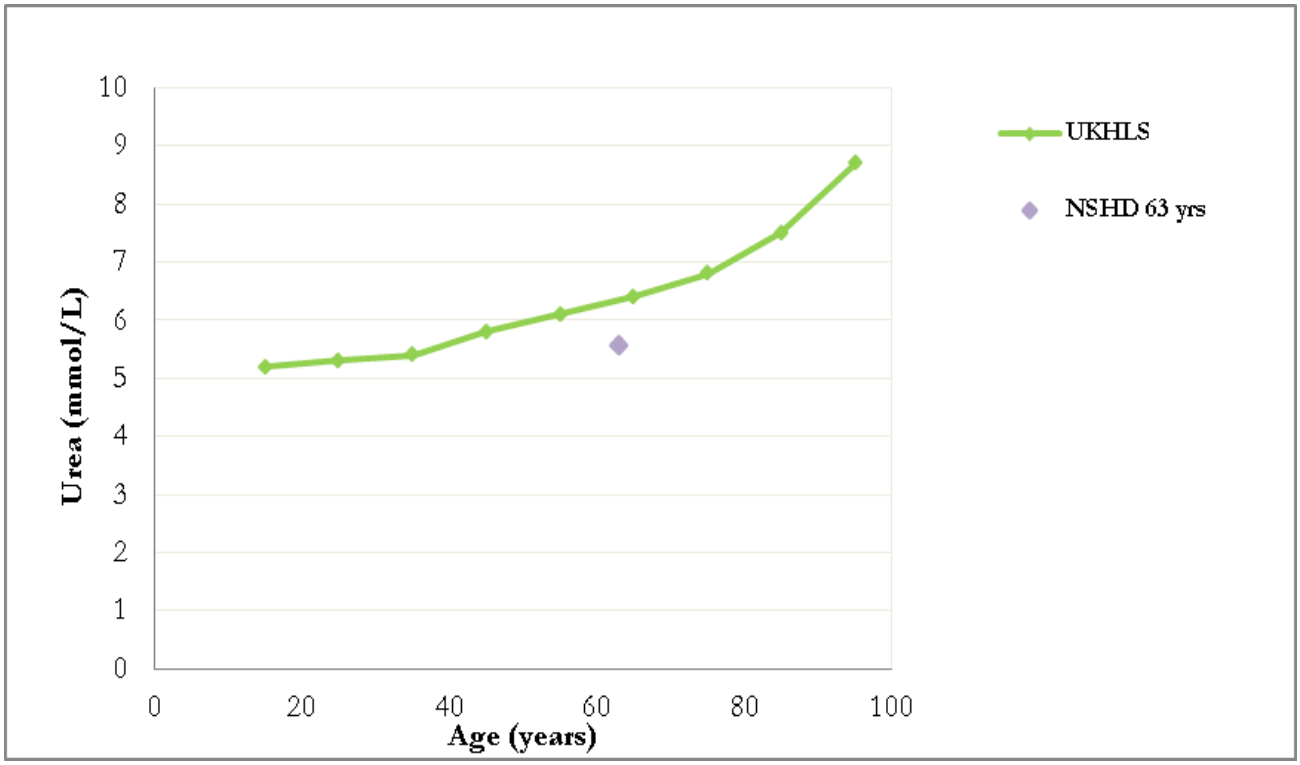
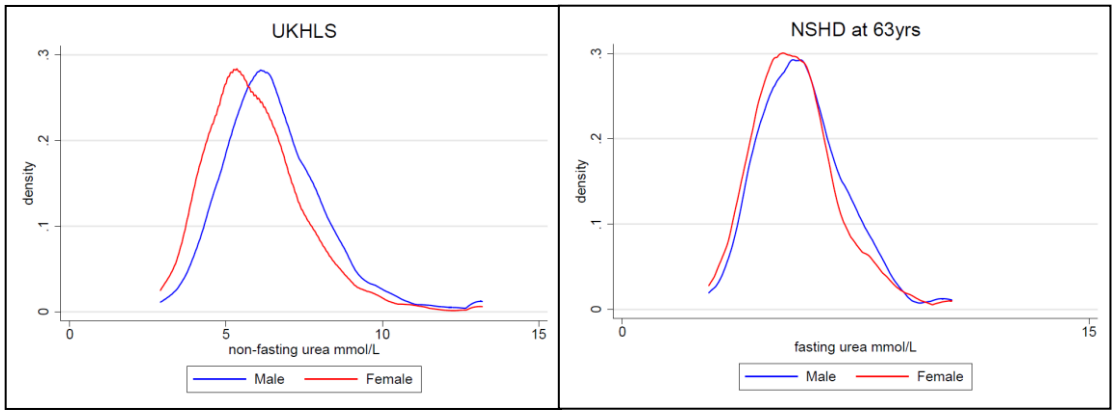


Figure B38. Distributions (Kernel density) of urea (mmol/L) by gender for each CLOSER study that measured urea. Distributions were winsorised at 0.5% and 99.5%.



Liver Function Tests (LFTs): ALT, ALP, AST

Liver function tests measure various chemicals in the blood made by the liver. Abnormal results may indicate a problem with the liver and may help to identify the cause.
The functions of the liver include: storing fuel for the body (called glycogen) which is made from sugars; helping to process fats and proteins from digested food; making proteins that are essential for blood to clot (clotting factors); processing of medications; helping to remove poisons and toxins from the body. The liver also makes bile. This is a greenish-yellow fluid that contains bile acids, bile pigments and waste products such as bilirubin.
As the liver performs its various functions, it makes chemicals that pass into the bloodstream. Various liver disorders alter the blood level of these chemicals. Some of these chemicals can be measured in a blood sample.
LFTs are some tests that are commonly done on a blood sample. These usually measure the following:

- **Alanine transaminase (ALT).**
- **Alkaline phosphatase (ALP).**
- **Aspartate aminotransferase (AST).**

ALT has been measured in three CLOSER studies: ALSPAC, UKHLS and NSHD; ALP has been measured in three CLOSER studies: UKHLS, SWS and NSHD; AST has been measured in three CLOSER studies: ALSPAC, UKHLS and NSHD (see **Table 2**).

What is it?

Alanine Transaminase (ALT) – an enzyme mainly found in the liver; the best test for detecting hepatitis, raised levels indicate liver damage

Alkaline Phosphatase (ALP) – an enzyme related to the bile ductus; often increased when they are blocked, either inside or outside the liver.

Aspartate Transaminase (AST) – an enzyme found in the liver and a few other places, particularly the heart and other muscles in the body, raised levels indicate liver damage

What is the clinical significance?

A panel of liver function tests, which can reflect how well the liver is functioning.

How is it measured?

ALT, ALP and AST are measured as described in **Table A39**, **Table A40** and **Table A41.**

Are there clinical cut points?

ALT and AST should be < 40 U/L. ALP should be between 30 and 130 IU/L for adults 20-70 yrs. For over 70 yrs., ALP should be between 30 and 150 IU/L.

What should be considered in analyses?

Recent alcohol intake influences the measures of these analytes. It is recommended that this is taken into account in analyses. Some medications may be associated with raised LFTs for example anti-epilepsy medications (BNF chapter 4.8) or statins (BNF chapter 2.12). Pregnancy can increase ALP

levels. Children have higher ALP levels because their bones are growing, and ALP is often very high during the 'growth spurt' which occurs at different ages in males and females. Temporary elevations of ALP are also seen with healing fractures. Transient benign increases in ALP may also be seen in young infants. Eating a meal can increase the ALP level slightly for a few hours in some people. Ideally the test should be done after fasting overnight. Some drugs may increase ALP levels, especially some of the drugs used to treat psychiatric problems or epilepsy but significant increases are rare.

Table A39. ALT (IU/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	17.5 yrs 3 258	Automated analyser with enzymatic methods	19.1 (11.1) 16.5 12.8-21.7	15.8 (8.4) 13.8 11.3-17.6
UKHLS	12 777	IFCC UV with Pyridoxal phosphate activation method on the Roche P module analyser	32.4 (16.4) 28.0 22.0-38.0	23.6 (12.2) 21.0 17.0-27.0
SWS	-	-	-	-
NCDS	-	-	-	-
NSHD	63 yrs 2 066	Colorimetrically on a Siemens Dimension Xpand analyser	32.7 (15.1) 29.0 23.0-38.0	26.6 (13.4) 24.0 19.0-30.0
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A39. Crude plot of ALT (IU/L), median, by age for the CLOSER studies that measured ALT.

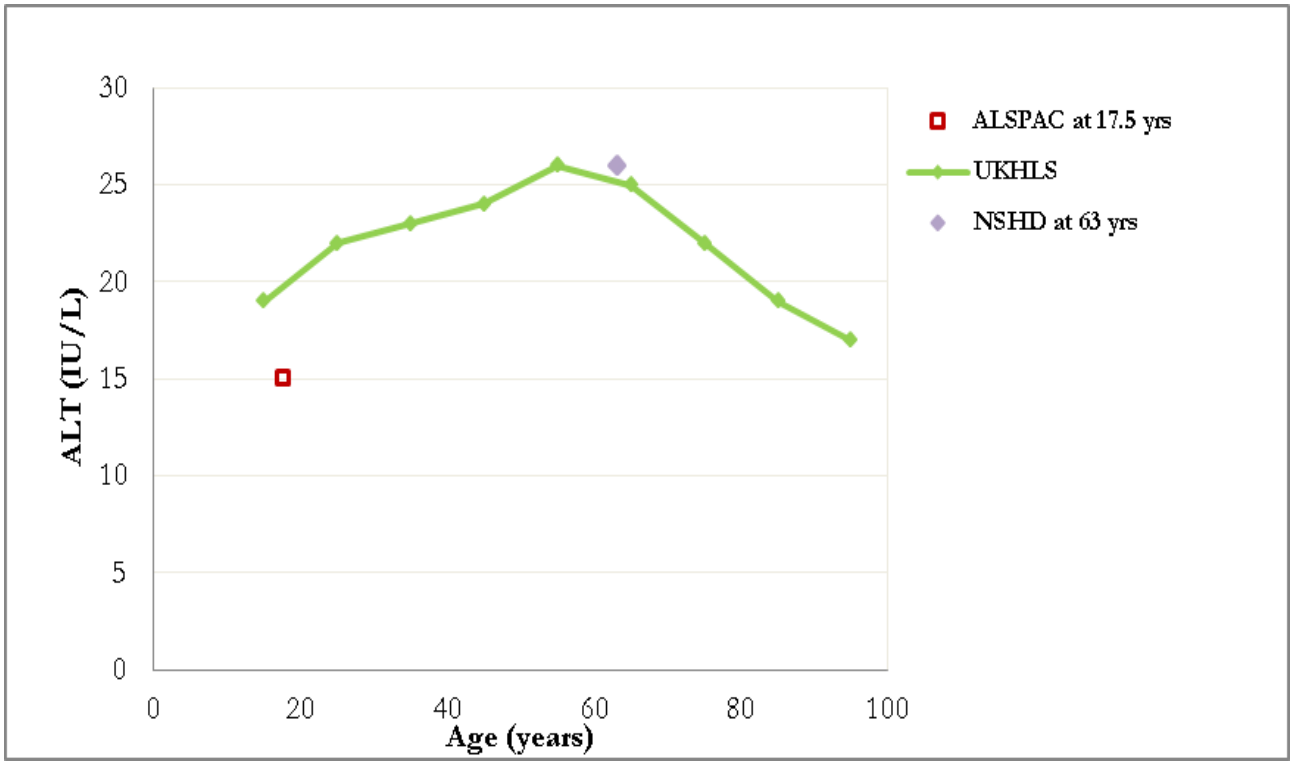


Figure B39. Distributions (Kernel density) of ALT (IU/L) by gender for each CLOSER study that measured ALT. Distributions were winsorised at 0.5% and 99.5%.

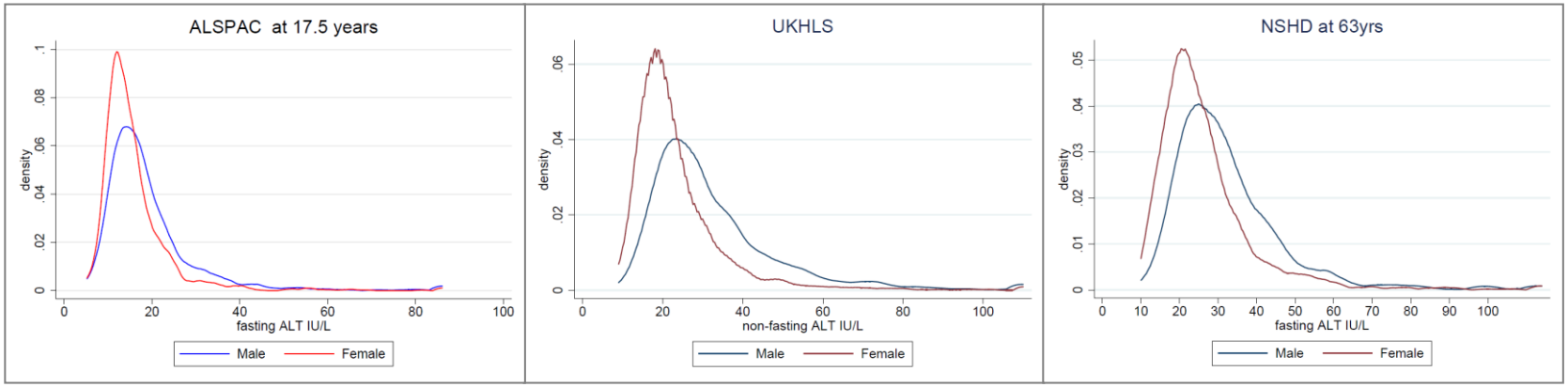


Table A40. ALP (IU/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%.

STUDY	Valid*, N	Method of Measurement	Mean (sd), Median, IQR	
			Males	Females
ALSPAC	-	-	-	-
UKHLS	12 785	IFCC UV with colourimetric PNP method on the Roche P module analyser	72.0 (20.1) 70.0 58.0-82.0	70.6 (20.9) 68.0 56.0-82.0
SWS	LP† 563		-	127.6 (1.5) 121.0 100.0-149.0
NCDS	-	-	-	-
NSHD	63 yrs 2 063	Colorimetrically on a Siemens Dimension Xpand analyser	78.7 (21.0) 76.0 65.0-90.0	85.9 (24.3) 83.0 69.0-98.0
HCS	-	-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

†LP: Approximate gestation age is 34 weeks.

Figure A40. Crude plot of ALP (IU/L), median, by age for the CLOSER studies that measured ALP.

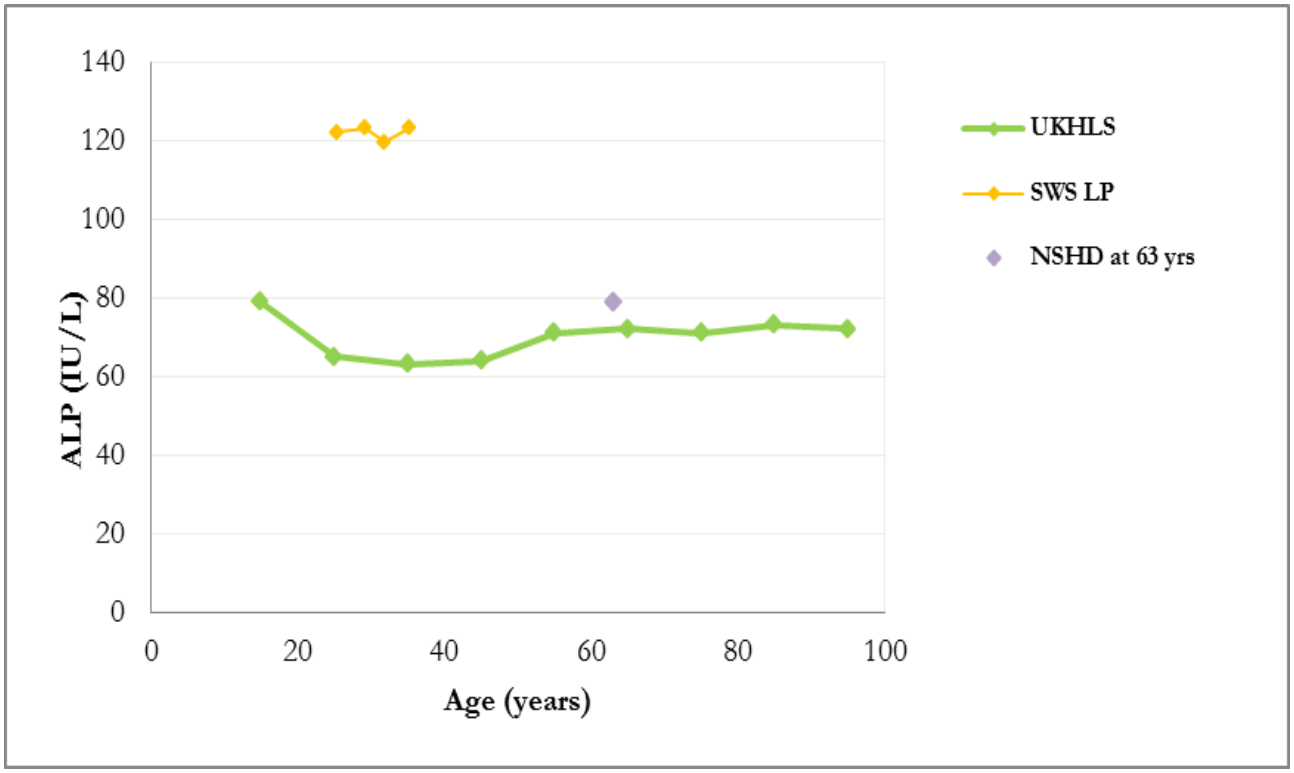


Figure B40. Distributions (Kernel density) of ALP (IU/L) by gender for each CLOSER study that measured ALP. Distributions were winsorised at 0.5% and 99.5%.

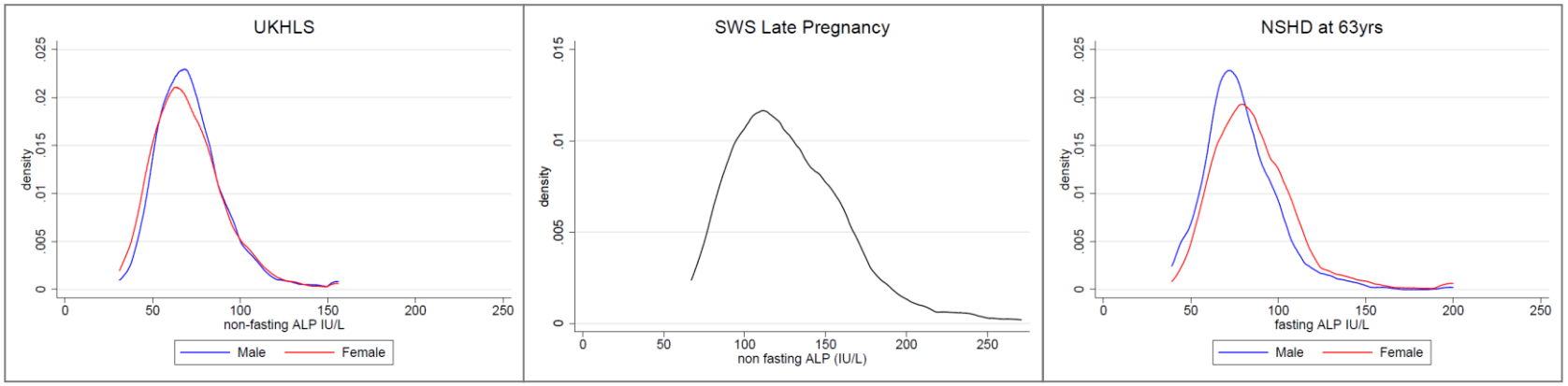


Table A41. AST (IU/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	17.5 yrs	3 258	Automated analyser with enzymatic methods.	22.7 (7.7) 21.1 18.0-25.2	19.4 (5.6) 18.4 16.2-21.3
UKHLS	12 386		IFCC UV with Pyridoxal phosphase activation method on the Roche P module analyser	32.7 (9.7) 31.0 27.0-36.0	28.4 (8.1) 27.0 24.0-31.0
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	2 066	Colorimetrically on a Siemens Dimension Xpand analyser	27.7 (1.0) 26.0 22.0-31.0	24.9 (8.5) 23.0 20.0-28.0
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A41. Crude plot of AST (IU/L), median) by age for the CLOSER studies that measured AST.

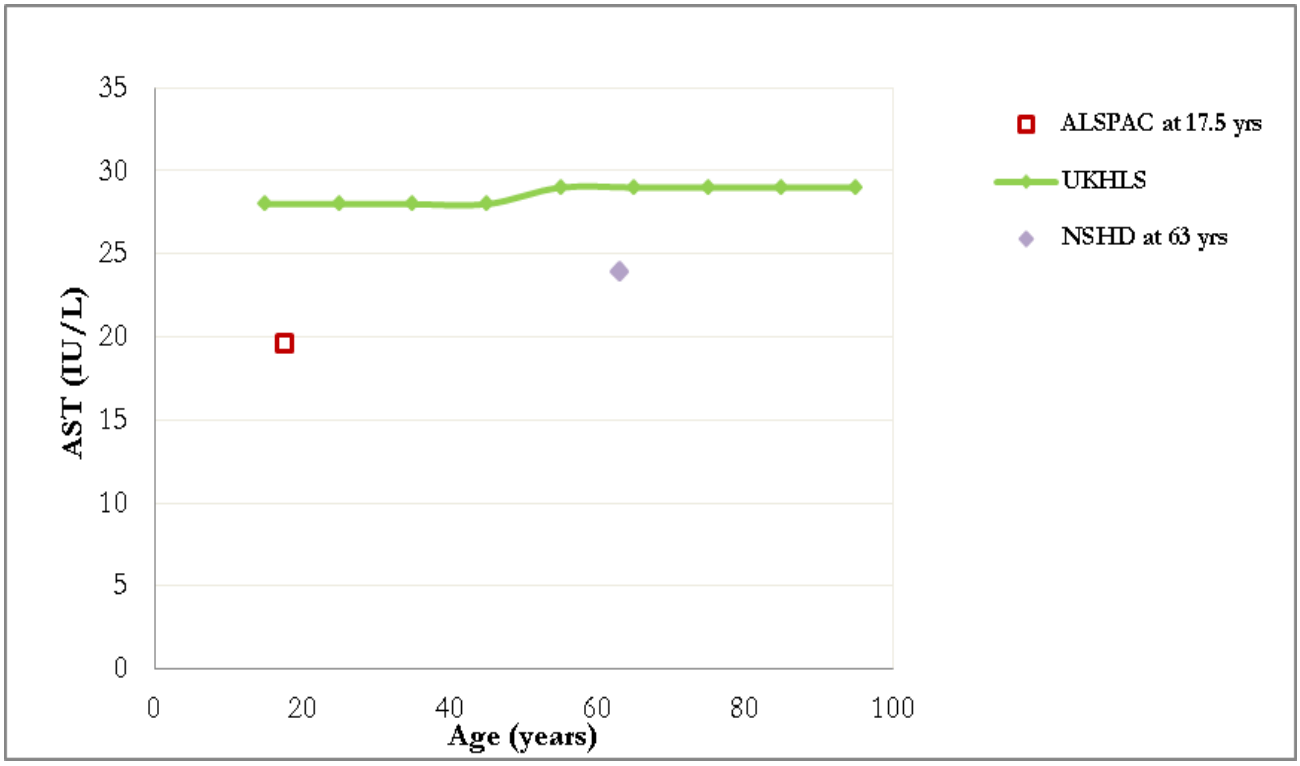
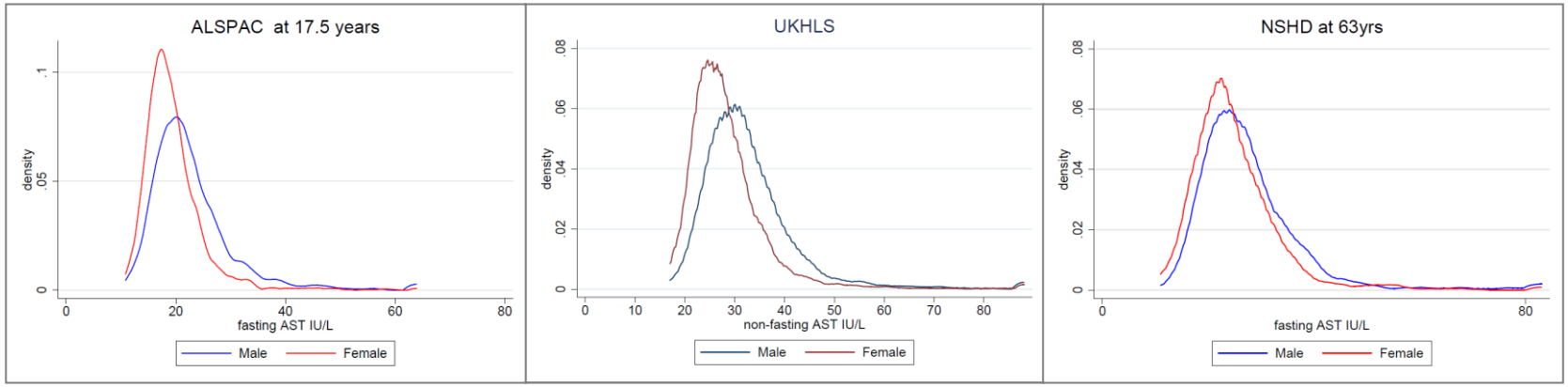


Figure B41. Distributions (Kernel density) of AST (IU/L) by gender for each CLOSER study that measured AST. Distributions were winsorised at 0.5% and 99.5%.



Albumin

Albumin has been measured in four CLOSER studies: ALSPAC, UKHLS, SWS and NSHD (see **Table 2**).

What is it?

Albumin measures the main protein made by the liver and tells us how well the liver is making this protein; low levels may indicate a loss of liver function.

What is its clinical significance?

Albumin is the most abundant protein in the blood plasma. It keeps fluid from leaking out of blood vessels; nourishes tissues; and carries hormones, vitamins, drugs, and ions like calcium throughout the body. Albumin is made in the liver and its concentration in the blood is sensitive to liver damage. The concentration of albumin in the blood drops when the liver is damaged or with a type of kidney disease called 'nephrotic' syndrome if a person experiences severe inflammation in the body, or with shock. The albumin concentration in the blood increases when a person is dehydrated.

How is it measured?

Albumin is measured as described in **Table A42**.

Are there clinical cut points?

Albumin should be >35g/L and <50 g/L.

What should be considered in analyses?

Recent alcohol intake influences the measures of albumin; it is recommended that this be taken into account in analyses. Certain drugs increase albumin in the blood, including anabolic steroids (BNF chapter 6.4.3), androgens (BNF chapter 8.3.3), growth hormones (BNF chapter 6.5.1), and insulin. Low albumin concentrations in the blood can also be seen in severe inflammation or shock. If a person is receiving large amounts of intravenous fluids, the albumin level may be inaccurate. Albumin will be decreased during pregnancy.

Table A42. Albumin (g/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	Pregnancy†	8 018	Standard laboratory methods on Roche Modular analysers	-	38.6 (5.4) 37.8 35.2-42.4
	7 yrs	1 860		41.1 (26.1) 35.2 25.3-49.3	48.7 (28.2) 41.3 29.1-60.5
	9 yrs	5 088		34.6 (17.7) 30.5 23.2-45.9	38.4 (19.2) 34.5 25.1-45.9
	11^{+} yrs	1 692		30.5 (17.9) 25.2 18.7-37.3	32.4 (17.8) 28.0 30.4-39.8
UKHLS	12 920		BCG colourimetric method on the Roche P module analyser	47.5 (3.0) 48.0 46.0-50.0	46.2 (2.7) 46.0 44.0-48.0
SWS	LP‡	565	Standard autoanalyser	-	30.0 (2.1) 30.2 28.6-31.6
NCDS	-		-	-	-
NSHD	63 yrs	2 066	Colorimetrically on a Siemens Dimension Xpand analyser	42.6 (2.9) 42.5 40.8-44.5	42.0 (2.7) 41.9 40-43.9
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**
† Approximate gestation age is 12-16 weeks
‡LP: Approximate gestation age is 34 weeks,

Figure A42. Crude plot of albumin (g/L), median, by age for the CLOSER studies that measured albumin.

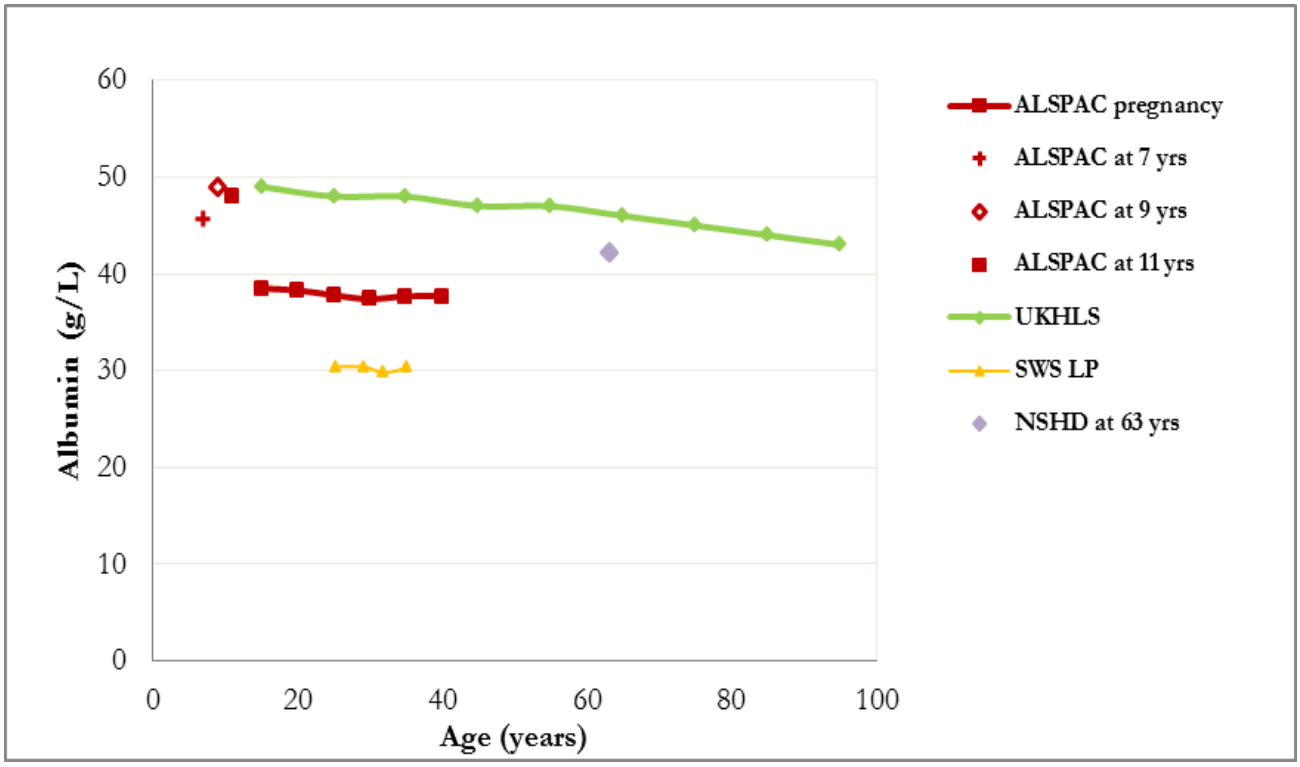
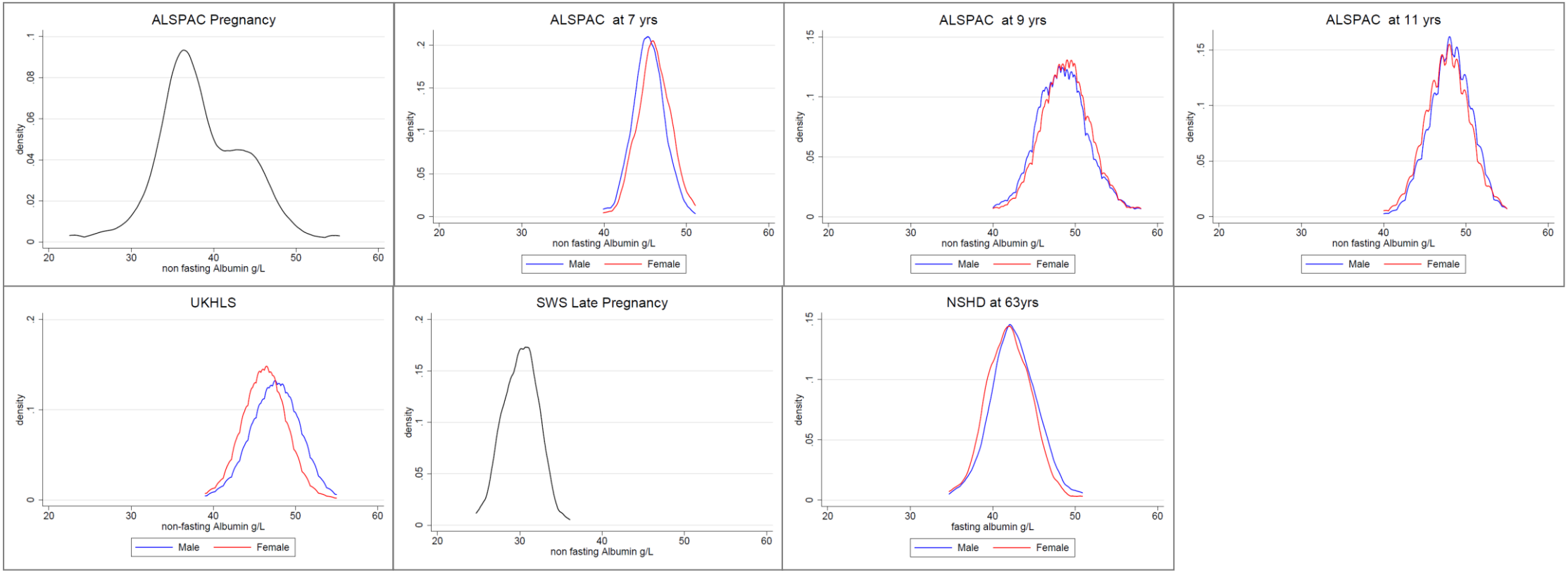


Figure B42. Distributions (Kernel density) of albumin (g/L) by gender for each CLOSER study that measured albumin. Distributions were winsorised at 0.5% and 99.5%.



Gamma Glutamyl Transferase (GGT)

GGT has been measured in three CLOSER studies: ALSPAC, UKHLS and NSHD (see **Table 2**).

What is it?

Gamma Glutamyl Transferase (GGT) is an enzyme found mainly in the liver. When the liver is injured or the flow of bile is obstructed, the concentration of GGT within the bloodstream rises. It is therefore a useful marker for liver disease and bile duct injury.

What is the clinical significance?

It measures how well the liver is functioning

How is it measured?

GGT is measured as described in **Table A43.**

Are there clinical cut points?

GGT should be <70 IU/L in males and <45 IU/L in females.

What should be considered in analyses?

Smoking and recent alcohol intake (within 24hrs of blood test) influences the measure of GGT. It is recommended that these be taken into account in analyses. Elevated concentrations may be due to congestive heart failure and liver disease. Use of non-prescriptions drugs such as nonsteroidal anti-inflammatory drugs (NSAIDs), lipid-lowering drugs (BNF chapter 2.12), antibiotics, histamine blockers (used to treat excess stomach acid production), antifungal agents, anticonvulsants (BNF chapter 4.8, seizure control medications), antidepressants (BNF chapter 4.3), and hormones such as testosterone can alter the levels of GGT. Oral contraceptives (BNF chapter 7.3, birth control pills) and clofibrate can decrease GGT concentrations.

Table A43. GGT (IU/L): Study, valid cases, laboratory method, mean (standard deviation), median and interquartile range (IQR). Distributions have been winsorised at 0.5% and 0.95%

STUDY	Valid*, N		Method of Measurement	Mean (sd), Median, IQR	
				Males	Females
ALSPAC	17.5 yrs	3 258	Automated analyser with enzymatic methods (University of Glasgow).	20.5 (8.7) 18.0 15.0-23.0	16.7 (7.2) 15.0 12.0-19.0
UKHLS	12 816		Enzymatic method on the Roche P module analyser	40.0 (38.4) 29.0 20.0-45.0	27.2 (28.5) 19.0 14.0-29.0
SWS	-		-	-	-
NCDS	-		-	-	-
NSHD	63 yrs	1 861	Colorimetrically on a Siemens Dimension Xpand analyser	34.8 (12.9) 32.8 25.0-42.0	27.4 (12.4) 23.5 18.3-32.6
HCS	-		-	-	-

*** Valid cases include all data not coded as missing, invalid, unavailable or similar.**

Figure A43. Crude plot of GGT (IU/L), median, by age for the CLOSER studies that measured GGT

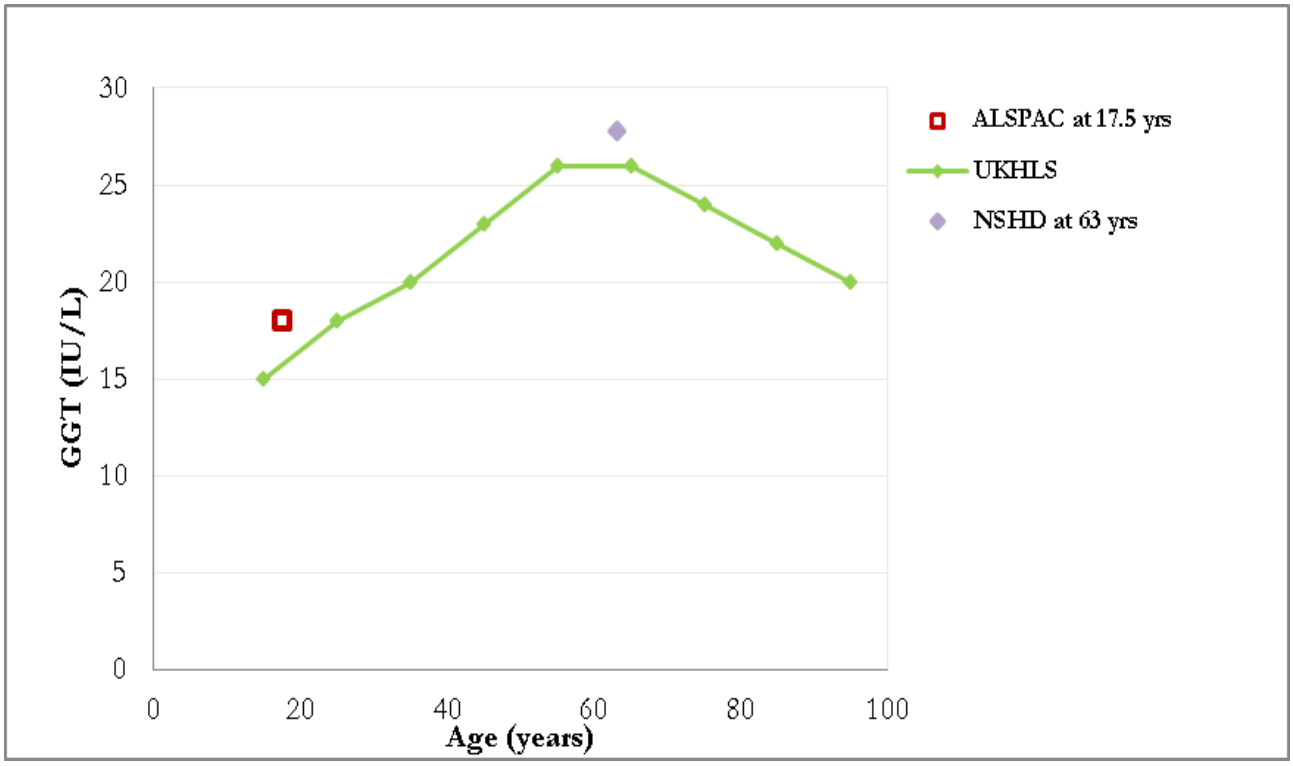
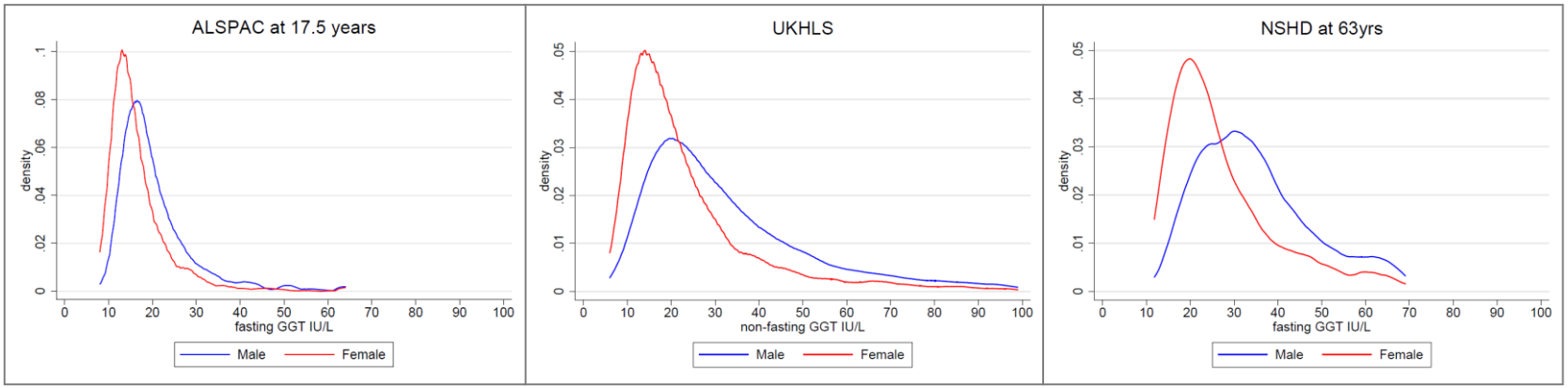


Figure B43. Distributions (Kernel density) of GGT (IU/L) by gender for each CLOSER study that measured blood GGT. Distributions were winsorised at 0.5% and 99.5%.



Conclusions

The United Kingdom is particularly strong in hosting a large number of richly characterised cohort and longitudinal studies that cover the entire life span from preconception to old age. This catalogue highlights the range of the biomarker data measured within the CLOSER studies.

This catalogue demonstrates that while data are available from participants across the age span, biomarker data in CLOSER are dominated by adults, and young people are under-represented. However, a number of physiological systems are represented by the biomarkers measured across the cohort and longitudinal studies, which should enable researchers to conduct cross-study analyses.

The purpose of the catalogue is to encourage such research. In particular it is hoped that the text accompanying the biomarker descriptions should alert researchers that are new to biomarker data to some of the issues to consider when conducting analyses. Further, given the strength of the social data available in the studies that make up CLOSER, it is anticipated that the biomarker data available and described in the catalogue should serve to address research questions that pertain to the biosocial research agenda.

Citation of the Catalogue:

Ruiz, Milagros; Benzeval, Michaela; Kumari, Meena. (2017). *Biomarkers in the studies included in CLOSER: a catalogue across cohort and longitudinal studies*. Colchester: University of Essex.