Blood biomarkers

BRHS 20 year follow-up (Q20)

1998 - 2000

Fasting blood samples

At the 20-year follow-up physical examination in 1998-2000 (Q20), the BRHS study participants were asked to fast for a minimum of 6 hours, during which they were instructed to drink only water and to attend for measurement at a prespecified time between 0800 and 1800 h. All men were asked to provide a blood sample, collected using the Sarstedt Monovette system. The samples were frozen and stored at −20°C on the day of collection and transferred in batches for storage at −70 °C until analysis, performed after no >1 freeze-thaw cycle.

The blood sampling procedure is described in section 4.2.6 and the handling of the blood samples is described in sections 5.1 to 5.5 of the 20-year follow-up physical examination protocol. (***BRHS 1998-2000 (Q20) 20yr follow-up Physical examination protocol.pdf***)

Blood marker adjustments

** Adjusted blood markers: A number of blood markers (Glucose, insulin, triglycerides and LDL) were adjusted for fasting duration (using the time the participants recorded as having something to eat or drink other than water (data from *question 20.0 in BRHS 1998-2000 20 year follow-up survey Main Qr Q20*). These blood markers are available both in unadjusted and adjusted form.

The method of adjustment is described in:

1. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501 and

2. BRHS document: *BRHS Q20 Blood marker Adjustment Report and SAS code by JEmberson.pdf* (Appendix 1)

A list of all BHRS bloods markers for 20-year follow-up in 1998-2000(Q20) can be found in the table below followed by the laboratory methods used. ** indicates the blood marker has been adjusted for fasting duration.

Appendix 1: BRHS Q20 Blood marker Adjustment Report and SAS code by JEmberson.pdf

Blood biomarkers - BRHS 1998-2000 (Q20) 20 year follow-up

Blood marker	units	Method section	BRHS variable name	Mean	SD	Min	Max	N	N Miss	Data access
Activated partial thromboplastin time (aPTT)	s	S.3	q20appt	30.98	3.63	22.4	79.4	4079	173	yes
Activated protein C (APC resistance)	ratio	S.3	q20apc	3.29	0.56	1.73	6.69	4050	202	yes
Adiponectin	µg/mL	S.5	q20adiponectin	8999.7	7319.7	6.15	66984.93	4046	206	yes
Alanine Transaminase (ALT)	u/l	S.1.3	q20alt	17.49	10.09	1	201	4034	218	yes
Albumin	g/l	S.1.6	q20alb	44.15	2.75	29	54	4034	218	yes
Alkaline Phosphatase (ALP)	u/l	S.1.5	q20alk_phos	85.82	41.56	16	1194	4034	218	yes
Aspartame Transaminase (AST)	u/l	S.1.3	q20ast	24.25	10.31	6	268	4034	218	yes
Bilirubin	µmol/L	S.1	q20bil	10.16	5.67	1	209	4021	231	yes
Calcium	mmol/L	S.1.6	q20ca	2.43	0.09	1.83	3.7	4033	219	yes
Carboxyhemoglobin	%	S.13	q20cohb	0.83	1.15	0	10.6	4025	227	yes
Cholesterol	mmol/l	S.1.1	q20chol	6	1.08	2.6	11.6	4031	221	yes
Copeptin	pmol/L	S.6	q20copeptin	5.43	5.29	0.89	91.91	3713	539	yes
Corrected Calcium	mmol/L	S.1.6	q20corr_ca	2.35	0.08	2.02	3.66	4033	219	yes
Cotinine	ng/ml	S.12	q20cotinine	41.96	107.49	0.05	844.2	3900	352	yes
C-Reactive protein (CRP)	mg/L	S.3	q20crp	3.53	6.86	0.18	120	4056	196	yes
Creatinine	µ mol/l	S.1	q20cre	98.15	21.19	39	531	4034	218	yes
Cysteine	mg/L	S.8	q20cys	312	37.91	165.2	484.8	3921	331	yes
Cysteinylglycine (CysGly)	µmol/L	S.8	q20cg	32.9	6.02	7.6	62.8	3921	331	yes
D-dimer	ng/ml	S.3	q20ddim	133.58	210.74	3	2852	4079	173	yes
eGFR $q20egfr=32788 \times q20cre^{(-1.154)} \times q20age^{(-0.203)}$		S.14	q20egfr	72.37	12.79	9.86	203.56	4034	218	yes
Factor IX	IU/dL	S.3	q20f9	131.3	25.85	19	223	4078	174	yes
Factor VII	IU/dL	S.3	q20f7	117.23	26.43	9	215	4073	179	yes
Factor VIII	IU/dL	S.3	q20f8	132.38	31.81	15	332	4077	175	yes
Fibrinogen	g/L	S.3	q20fib	3.27	0.74	0.49	8.16	4080	172	yes
Nephelometric fibrinogen	g/L	S.3	q20nephfib	3.17	0.72	0	8.59	4026	226	yes
Gamma-glutamyl transferase (gamma-GT, GGT)	u/l	S.1.3	q20ggt	36.83	55.05	7	1879	4034	218	yes
**Glucose(adjusted)	mmol/l	S.1.2a	q20dglu	6.03	1.89	2.55	27.6	4032	220	yes
Glucose(unadjusted)	mmol/l	S.1.2	q20glu	6.06	1.91	2.5	27.6	4032	220	yes
Glycated Haemoglobin (HbA1c)	%	S.15.1	q20hba1c	5.03	0.93	1.2	12.9	4039	213	yes
Haematocrit	l/l	S.15	q20hct	0.45	0.03	0.28	0.66	4036	216	yes
Haemoglobin(hb)	g/l	S.15	q20hb	14.58	1.18	7.1	20.2	4036	216	yes
HDL cholesterol	mmol/l	S.1.1	q20hdl	1.32	0.34	0.3	3.3	4006	246	yes

** blood marker has been adjusted for fasting duration

Blood marker/cont.	units	Method section	BRHS variable name	Mean	SD	Min	Max	N	N Miss	Data access
Homocysteine	µmol/L	S.8	q20hcy	13.48	6.47	5.3	128.7	4021	231	yes
**Insulin(adjusted)	pmol/L	S.2a	q20dinsul	11.19	14.89	1.12	522	4023	229	yes
Insulin(unadjusted)	pmol/L	S.2	q20rinsul	10.93	15.11	1	522	4023	229	yes
Interleukin-6 (IL-6)	pg/ml	S.3	q20il6	3.18	2.95	0.06	16	4050	202	yes
Leptin	ng/ml	S.7	q20leptin	12.34	13.05	0	173.7	4018	234	yes
**LDL cholesterol(adjusted)	mmol/l	S.1.1a	q20dldl	3.89	0.97	0.71	9.37	3974	278	yes
LDL cholesterol(unadjusted)	mmol/l	S.1.1	q20ldl	3.87	0.97	0.71	9.1	3974	278	yes
Magnesium (Mg)	mmol/L	S.1.6	q20mg	0.81	0.07	0.48	1.06	4031	221	yes
Mean Cell Haemoglobin Concentration (MCHC)	g/dl	S.15	q20mchc	32.37	1.19	25.84	37.11	4036	216	yes
Mean Cell Haemoglobin (MCH)	pg	S.15	q20mch	30.08	1.84	17	40	4037	215	yes
Mean Cell volume (MCV)	fl	S.15	q20mcv	92.97	5.27	65.7	123.1	4036	216	yes
Mean Platelet volume (MPV)	fl	S.15	q20mpv	8.4	1.73	3.6	14.9	3889	363	yes
MR-proADM	nmol/L	S.4	q20MRproADM	0.6	0.21	0.05	6.24	4050	202	yes
NT-proBNP	pg/ml	S.4	q20bnp	238.29	535.72	5	9771	3784	468	yes
Parathyroid Hormone (Pth)	pg/mL	S.11	q20PTH	48.56	21.27	14.5	620.6	3889	363	yes
Phosphate	mmol/l	S.1.5	q20phos	1.16	0.16	0.47	2.1	4012	240	yes
Plasma Viscocity	mPa s	S.3	q20pv	1.29	0.08	1.1	2.33	4013	239	yes
Platelets	10^9 /l	S.15	q20plts	235.58	63.44	27	899	4036	216	yes
Potassium (K)	mmol/l	S.1	q20k	4.43	0.37	3.1	6.3	4013	239	yes
Protein	g/l	S.1	q20prot	76.96	4.7	56	143	4034	218	yes
Red blood cell count (RBC)	10^12 /l	S.15	q20rbc	4.86	0.4	2.56	6.7	4036	216	yes
Sodium (Na)	mmol/l	S.1.4	q20na	139.62	2.74	125	150	4034	218	yes
Tissue plasminogen activator(tPA)	ng/ml	S.3	q20tpa	11.08	4.44	1.5	36.8	4083	169	yes
**Triglycerides(adjusted)	mmol/l	S.1.1a	q20dtrig	1.86	1.08	0.37	16.66	4032	220	yes
Triglycerides(unadjusted)	mmol/l	S.1.1	q20trigs	1.84	1.09	0.35	15.95	4032	220	yes
Troponin T(TnT)	pg/ml	S.4	q20TNT	14.11	22.25	2.99	1301	4046	206	yes
Urate	mmol/l	S.1.7	q20urate	0.38	0.08	0.14	0.8	4034	218	yes
Urea	mmol/l	S.1	q20urea	6.05	1.7	1.8	24.3	4034	218	yes
Vitamin C	µmol/L	S.9	q20vit_c	30.22	27.35	0	447.92	3948	304	yes
Vitamin D,	nmol/L	S.10	q20Total_VitD	20.01	9.24	2	120	3799	453	yes
Vitamin E	mmol/L	S.9	q20vit_e	33.59	11.95	0.64	118.65	3779	473	yes
Von Willebrand factor (VWF)	IU/ml	S.3	q20vwf	139.96	46.19	23	382	4083	169	yes
White blood cell count (WBC)	10^9/L	S.15	q20wbc	7.11	2.18	2.7	38.6	4037	215	yes

** blood marker has been adjusted for fasting duration

S.0 Blood biomarkers - Laboratory methods and reference papers

S.1 Biochemistry variables

Measured at the Department of Chemical Pathology, Royal Free Hospital, London (Prof Mike Thomas).
Biochemistry variables including S.1.1 – S.1.7 were measured using a Hitachi 747 automated analyser.

S.1.1 Lipids

All blood samples were separated and frozen on the day of collection and transferred to central laboratories for analysis.

Total cholesterol, HDL cholesterol and Triglyceride were measured using a Hitachi 747 automated analyser. Total and HDL cholesterol were analysed using the methods of Siedel [1] and Sugichi [2] respectively. **LDL-cholesterol** values were calculated using the Friedrickson-Friedwald equation.

1. Siedel J, Hagele EO, Ziegenhorn J, Wahlefield AW. Reagent for the enzymatic determination of serum total with improved lipolytic efficiency. Clin Chemistry 1983; 29: 1075-1080.
2. Sugishi H, Uji Y, Okabe H, Uekema K, Kjayahar N. Direct measurement of high-density lipoprotein cholesterol in serum with polyethylene glycol modified enzymes and sulphated alpha-cyclodextrin. Clin Chemistry 1995; 41: 717-723.
3. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501

S.1.1a ** Triglyceride and **LDL (adjusted for for differences between participant's fasting duration)

1. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501
2. BRHS document: BRHS Q20 Blood marker Adjustment Report and SAS code by JEmberson.pdf

S.1.2 Glucose

Plasma glucose was measured using the method of Trinder [1] using a Falcor 600 automated analyser.

1. Trinder P. Determination of blood glucose using 4-aminophenazone as oxygen acceptor. J Clin Pathology 1969; 22: 246.
2. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501

S.1.2a **Glucose (adjusted for for differences between participant's fasting duration)

1 Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501

2 BRHS document: BRHS Q20 Blood marker Adjustment Report and SAS code by JEmberson.pdf

S.1.3 Hepatic enzymes

Hepatic enzymes including GGT, ALT and AST, were measured using a Hitachi 747 automated analyzer.

Gamma-GT

A commercial assay supplied by Roche Diagnostics for use on Hitachi analysers. The method is standardised against the original kinetic method of Szasz but uses a water-soluble substrate L-gamma-glutamyl-3-carboxy-4-nitroanilide. Gamma-glutamyltransferase transfers the gamma-glutamyl group of this substrate to glycylglycine. the amount of 5-amino-2-nitrobenzoate liberated is proportional to the GGT activity and can be determined spectrophotometrically.

1. Szasz, G, Persjin, JP et.al. Z Klin Chem Klin Biochem (1974) 12 : 228.
2. Persijn JP, van der Slik W. A new method for the determination of gamma-glutamyltransferase in serum. J Clin Chem Clin Biochem. 1976 Sep;14(9):421-7.

ALT, AST

A commercial assay supplied by Roche Diagnostics for use on Hitachi analysers. The method is derived from the recommended IFCC reference method. The enzyme ALT catalyses the equilibrium reaction between alpha-ketoglutarate and L-alanine with that of L-glutamate and pyruvate. The increase in pyruvate is determined in an indicator reaction catalysed by lactate dehydrogenase in which NADH is oxidised to NAD+. The rate of the photometrically determined NADH decrease is directly proportional to the rate of formation of pyruvate and hence the ALT activity.

1. Bergmeyer HU, Horder M, Rej R. International Federation of Clinical Chemistry (IFCC) Scientific Committee, Analytical Section: approved recommendation (1985) on IFCC methods for the measurement of catalytic concentration of enzymes. Part 3. IFCC method for alanine aminotransferase (L-alanine: 2-oxoglutarate aminotransferase, EC 2.6.1.2). J Clin Chem Clin Biochem. 1986 Jul;24(7):481-95.

S.1.4 Sodium

Sodium was measured by an ion selective electrode.

1. Wannamethee SG, Shaper AG, Lennon L, Papacosta O, Whincup P. Mild hyponatremia, hypernatremia and incident cardiovascular disease and mortality in older men: A population-based cohort study.Nutr Metab Cardiovasc Dis. 2016 Jan;26(1):12-9

S.1.5 Phosphate, Alkaline Phosphatase (ALP)

Serum phosphate and ALP were both analysed on a Hitachi autoanalyser using colorimetric assays (Roche). The serum phosphate assay was based on the detection of ammonium phosphomolybdate; the ALP assay was based on the detection of p-nitrophenol released by ALP activity.

1. Wannamethee SG, Sattar N, Papacosta O, Lennon L, Whincup PH. Alkaline phosphatase, serum phosphate, and incident cardiovascular disease and total mortality in older men. Arterioscler Thromb Vasc Biol. 2013 May;33(5):1070-6.

S.1.6 Magnesium, Albumin, Calcium

Measured with an enzymatic colorimetric assay using a Hitachi 747 automated analyser.

S.1.7 Urate

Urate was measured with an enzymatic colorimetric assay using a Hitachi 747 automated analyser.

S.2 Insulin

Measured at the Department of Diabetes, University of Newcastle-on-Tyne (Prof G Alberti)
Serum insulin was measured using an enzyme-linked immunosorbent assay ELISA which does not cross-react with proinsulin. [1]

1. Andersen L, Dinesen B, Jorgensen PN, Poulsen F, Roder ME. Enzyme immunoassay for intact human insulin in serum or plasma. Clin Chemistry 1993; 39: 578-582.
2. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501

S.2a ** Insulin (adjusted for differences between participant's fasting duration)

1. Emberson J,Whincup PH, Walker M , Thomas M,Alberti KGMM. Biochemical measures in a population-based study: effect of fasting duration and time of day. Ann Clin Biochem 2002; 39: 493- 501
2. **BRHS document: BRHS Q20 Blood marker Adjustment Report and SAS code by JEmberson.pdf**

S.3 Haemostatic and inflammatory variables

Measured at the University of Glasgow (Prof G Lowe)

Haemostatic: aPTT, APC, D-dimer, Factor VII, Factor VIII, Factor IX, tPA, vWf
Inflammatory: Fibrinogen, Nephelometric fibrinogen, Plasma viscosity, CRP, IL-6

At the 20-year examination, blood was anticoagulated with K2 EDTA (1.5 mg mL−1) for measurement of plasma viscosity at 37 °C in a semi-automated capillary viscometer (Coulter Electronics, High Wycombe, UK). Blood was also anticoagulated with 0.109 m trisodium citrate (9:1 v:v) for measurement of clottable fibrinogen (Clauss method); as well as coagulation factors (F)VII, VIII and IX; activated partial thromboplastin time (APTT) and activated protein C (APC) ratio (measured by the APTT-based method) in an MDA-180 coagulometer (Organon Teknika, Cambridge, UK). Plasma levels of t-PA antigen and D-dimer were measured with enzyme-linked immunosorbent assays (ELISA) (Biopool AB, Umea, Sweden) as was VWF antigen (Dako, High Wycombe, UK). C-reactive protein (CRP) was assayed by ultra-sensitive nephelometry (Dade Behring, Milton Keynes, UK). IL-6 was assayed using a high-sensitivity ELISA (R & D Systems, Oxford, UK). [1]

1. Wannamethee SG, Whincup PH, Shaper AG, Rumley A, Lennon L, Lowe GD. Circulating inflammatory and hemostatic biomarkers are associated with risk of myocardial infarction and coronary death, but not angina pectoris, in older men. J Thromb Haemost. 2009 Oct;7(10):1605-11.
2. Rumley A, Emberson JR, Wannamethee SG, Lennon L, Whincup PH, Lowe GD. Effects of older age on fibrin D-dimer, C-reactive protein, and other hemostatic and inflammatory variables in men aged 60-79 years. J Thromb Haemost. 2006 May;4(5):982-7.

S.4 Cardiac markers

Measured at the University of Glasgow (Dr Paul Welsh)

NT-proBNP, hsTnT, and MR-proADM

NT-proBNP and hsTnT were measured in plasma samples on an automated clinically validated immunoassay analyzer (e411, Roche Diagnostics, Burgess Hill, United Kingdom) using the manufacturers' calibrators and quality control reagents. MR-proADM was measured on an automated B.R.A.H.M.S Kryptor Compact plus (Thermo Fisher Scientific Hemel Hempstead, United Kingdom).[1]

1. Welsh P, Hart C, Papacosta O, Preiss D, McConnachie A, Murray H, et al. Prediction of Cardiovascular Disease Risk by Cardiac Biomarkers in 2 United Kingdom Cohort Studies: Does Utility Depend on Risk Thresholds For Treatment? Hypertension. 2016;67(2):309-15. Epub 2015/12/17.

Other blood markers

S.5 Adiponectin

Measured at the University of Glasgow (Prof Naveed Sattar)

1. Sattar N, Wannamethee G, Sarwar N, Tchernova J, Cherry L, Wallace AM, Danesh J, Whincup PH. Adiponectin and coronary heart disease: a prospective study and meta-analysis.Circulation. 2006 Aug 15;114(7):623-9.

S.6 Copeptin

Measured at the University of Glasgow (Prof Naveed Sattar)

1. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup PH, Sattar N. Copeptin, Insulin Resistance, and Risk of Incident Diabetes in Older Men. Clin Endocrinol Metab. 2015 Sep;100(9):3332-9.

S.7 Leptin

Measured at the University of Glasgow (Prof Naveed Sattar)

1. Sattar N, Wannamethee G, Sarwar N, Chernova J, Lawlor DA, Kelly A, Wallace AM, Danesh J, Whincup PH. Leptin and coronary heart disease: prospective study and systematic review. J Am Coll Cardiol. 2009 Jan 13;53(2):167-75.

S.8 Homocysteine, Cysteine and Cysteinyl-glycine

Measured in Tromso, Norway (Dr Ueland)

S.9 Plasma Vitamin C and Vitamin E

Measured at the Department of Biochemistry, Royal Free Hospital, London (Prof Bruckdorfer)

Plasma vitamin C and vitamin E were measured with high performance liquid chromatography using ultraviolet and fluorescent detection in plasma. For vitamin C, plasma extracts were treated with metaphosphoric acid at the point of collection and were then snap frozen with dry ice (1,2).

1. Rice-Evans, Diplock CAA, Symons MCR. Techniques in free radical research. In: Burdon RH, Vanknippenberg PH, eds. Laboratory techniques in biochemistry and molecular biology. Amsterdam: Elsevier, 1991: 185–206.
2. Jennings PE, Chirico S, Jones AF, Lunec J, Barnett AH. Vitamin C metabolites and microangiopathy in diabetes mellitus. Diabetes Res 1987; 6: 151–4.
3. Wannamethee SG, Bruckdorfer KR, Shaper AG, Papacosta O, Lennon L, Whincup PH. Plasma vitamin C, but not vitamin E, is associated with reduced risk of heart failure in older men. Circ Heart Fail. 2013 Jul;6(4):647-54.

S.10 Total vitamin D

Measured at the University of Glasgow (Prof Naveed Sattar)

Total vitamin D (25OHD2 plus 25OHD3) was measured using a gold-standard liquid chromatography–tandem mass spectrometry method following an automated solid-phase extraction procedure [1]. Measurements were made in ng/ml and converted into nmol/l. The lower limit of sensitivity was 10 nmol/l. PTH was measured by electrochemiluminescence using a clinically validated assay for intact PTH [1].

1. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup Peter H, Sattar N. Elevated parathyroid hormone, but not vitamin D deficiency, is associated with increased risk of heart failure in older men with and without cardiovascular disease. Circ Heart Fail 2014; 7: 732–9.

S.11 Parathyroid hormone (PTH)

Measured at the University of Glasgow (Prof Naveed Sattar)

Plasma PTH was measured by electrochemiluminescence using a clinically validated assay for intact PTH on the Elecsys 2010 (Roche Diagnostics) using the manufacturer's calibrators and controls.[1]

1. Wannamethee SG, Welsh P, Papacosta O, Lennon L, Whincup Peter H, Sattar N. Elevated parathyroid hormone, but not vitamin D deficiency, is associated with increased risk of heart failure in older men with and without cardiovascular disease. Circ Heart Fail 2014; 7: 732–9.

S.12 Cotinine

Measured at New Cross Hospital, London (C Feyerabend)

Liquid chromatography tandem mass spectrometry was used to assay cotinine in serum samples [1]

1. Jefferis BJ, Lowe GD, Welsh P, Rumley A, Lawlor DA, Ebrahim S, Carson C, Doig M, Feyerabend C, McMeekin L, Wannamethee SG, Cook DG, Whincup PH. Second hand smoke (SHS) exposure is associated with circulating markers of inflammation and endothelial function in adult men and women. .Atherosclerosis. 2010 Feb;208(2):550-6.

S.13 Carboxyhaemoglobin

Measured at the Whittington Hospital, London.

COHb was measured using a co-oximeter (AVL Medical Instruments, Ltd) which was calibrated with each batch of samples and was registered in an external quality assurance programme.

Whincup PH, Papacosta O, Lennon L, Haines A . Carboxyhaemoglobin levels and their determinants in older British men BMC Public Health. 2006 Jul 18;6:189.

S.14 eGFR

eGFR(q20egfr) =32788 x q20cre $^{(-1.154)}$ x q20age $^{(-0.203)}$ where q20cre **is creatinine** and q20age is **age**

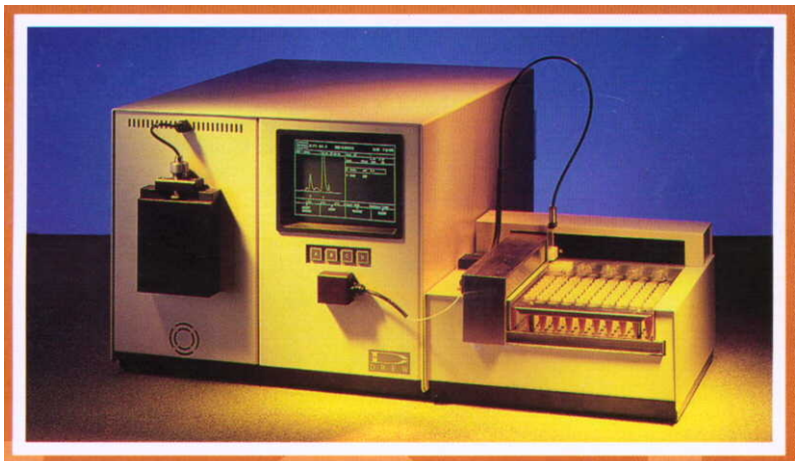
S.15 Routine Haematology

Routine haematology analyses were carried out at the Dept of Haematology, Whittington Hospital, London.

Blood was anticoagulated with K_2 EDTA (1.5 mg mL^{-1}) for measurement of haematocrit, white cell count, and platelet count in an automated cell counter.

S.15.1 Glycated Haemoglobin (HbA1c)

Drew Scientific HbGold Analyser



Intended use

The Drew Scientific HbGold Analyser, associated reagent kit and controls are intended for the in vitro measurement of HbA1c, HbA2 and the identification of haemoglobin variants in EDTA anticoagulated whole blood samples.

Principle

The instrument utilises automated cation exchange chromatography in conjunction with gradient elution to separate human haemoglobin subtypes and variants from haemolysed whole blood.

Results are automatically displayed on an integral screen as a chromatogram and they can be also be stored by an internal computer, transmitted by a standard RS232 port or printed on the standard dot-matrix printer supplied. Data can also be archived to floppy disk.

System

The Drew Scientific HbGold Analyser includes a 100 position cooled Autosampler which handles pre-diluted blood samples and automatically introduces them to the analyser.

For HbA1c measurement the analysis time is 5.5 minutes, for HbA2 and variants is 7 minutes and there is a 13-minute high-resolution assay available for further haemoglobin investigations.

The software allows the user to change between the above assays even within the same batch, using a single “Gold” column and reagent kit.

Precision

The maximum co-efficient of variance is 5% for the HbA1c and HbA2 assays.

Wed. 25 April 2000 - Wed. 27 June 2000

Adjustment of Continuous Variables for Fasting Duration and Time of Appointment.

Jon Emberson – 8/3/2001

SUMMARY

An adjustment for differences between subject's fasting durations was carried out for total cholesterol, LDL cholesterol, triglycerides, insulin and glucose. A cut off point of 6 hours was used to define a successful faster from a non successful one. For total cholesterol, non-fasters had their total cholesterol values adjusted upwards by an amount based on their diabetic status only. For LDL cholesterol and triglycerides, diabetics and non-diabetics were adjusted separately based on the fitting of two separate models. In both cases, the non fasters were adjusted to amount synonymous with the fasters. For insulin and glucose levels, it became apparent that there was a diurnal effect of fasting time, with subjects with either a short (<6 hours) or medium duration fast (14 hours) having markedly higher readings than other subjects. This was almost certainly due to miss-reporting of true fasting duration times by subjects whose appointment was in the afternoon. Therefore, for these responses, models were built based on *both* fasting duration and time of appointment. All subjects were subsequently standardised to that of a 6 hour faster with a 1P.M appointment. Again, models were fitted separately for both diabetics and non-diabetics.

VARIABLES OF INTEREST

The variables considered to potentially vary by fasting duration times were total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides, fibrinogen, insulin and glucose. At the onset, diabetics were considered separately from non-diabetics, as they were the only group of subjects that were not asked to fast for a period of at least six hours before their examination.

TRANSFORMATION OF VARIABLES

All the measures apart from total cholesterol were skewed to some degree. The square root transformation was applied to HDL and LDL cholesterol, and to fibrinogen, and the log transform applied to triglycerides and insulin. A double log transformation was applied to glucose levels, due to the extent of skewness observed in this data.

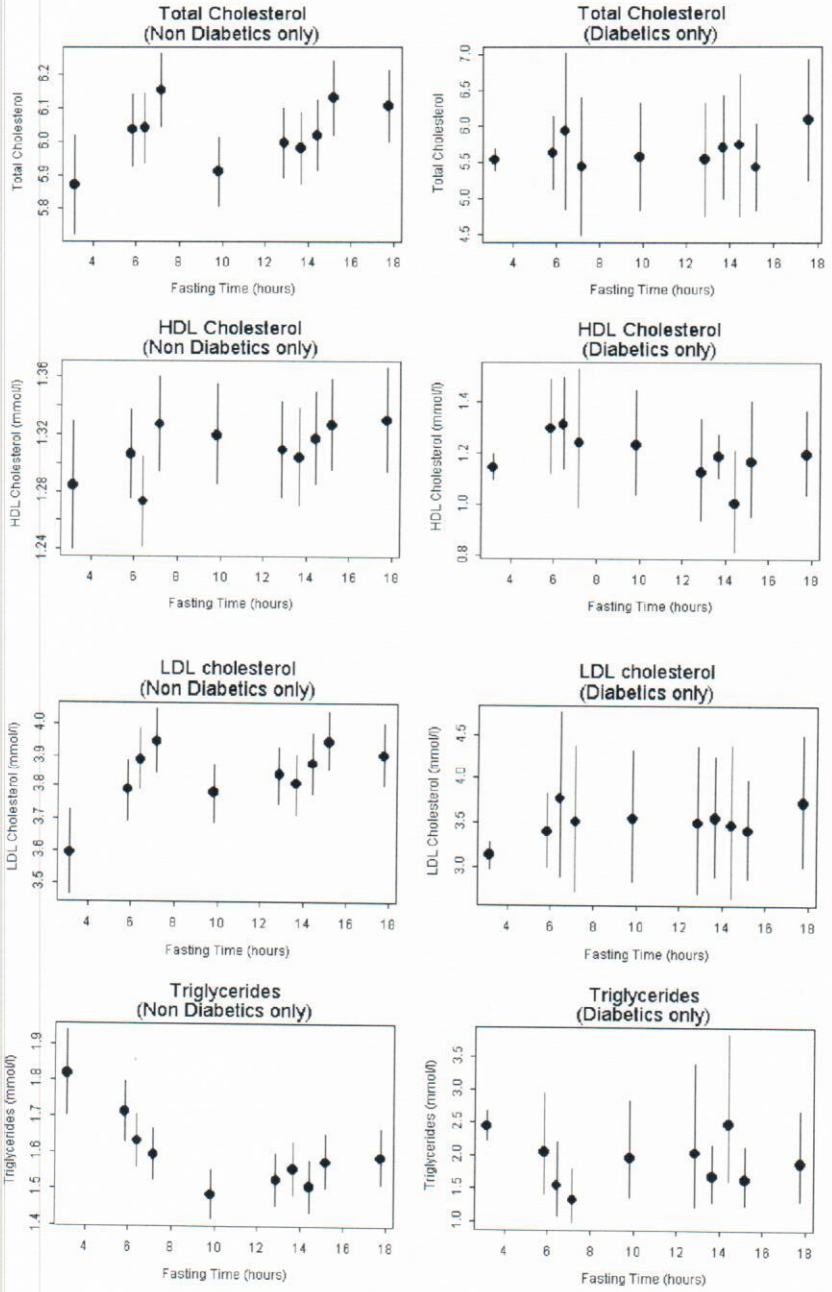
CALCULATION OF FASTING TIMES

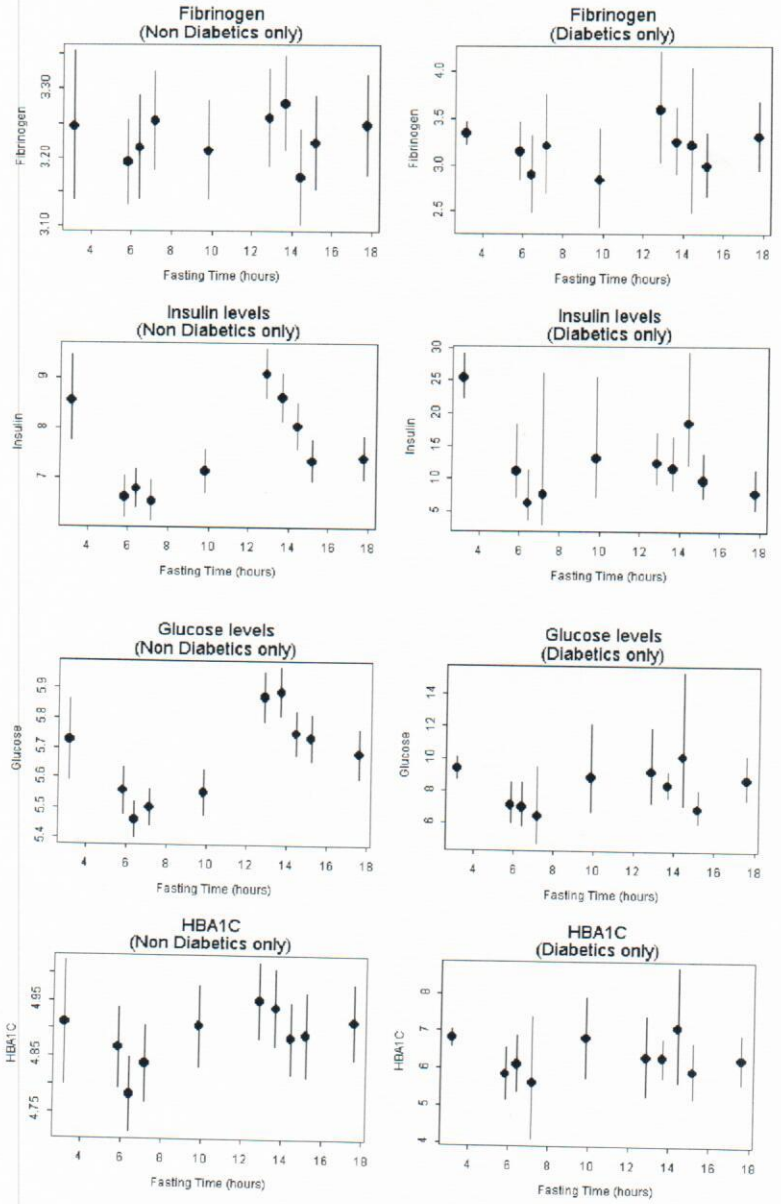
Fasting duration times were calculated for each subject attending the 20 year follow up as the difference in hours from the time recorded on the datasheet (when the sample was taken) and the time that the subject claimed to have last eaten (taken from the blue questionnaire). In cases where the subject last ate yesterday, but gave no actual time estimate, the time was estimated as 9pm, and the fasting time was calculated accordingly.

EXPLORATORY ANALYSIS – RELATIONSHIPS WITH FASTING DURATION

Fasting times were available for 4220 of the 4252 subjects that attended the twenty year follow up. Fasting times ranged from 5 minutes to 33 hours. The mean (standard deviation) fasting time for diabetics was 6.3 hours (5.5 hours), and was 11.1 hours (4.7 hours) for non-diabetics. 87% of non diabetics successfully fasted for the requested period of 6 hours. 33% of diabetics claimed to have fasted for at least 6 hours.

Subjects were categorized into groups defined by the deciles of fasting duration. For each group and each measure, 95% confidence intervals for the mean were calculated and plotted in an attempt to view any possible threshold relationships with levels of fasting duration. These are shown below and overleaf for both diabetics (right) and non-diabetics (left).





ANALYSIS BY FASTING DURATION AND DIABETIC STATUS: 2-WAY ANOVA

Preliminary analyses were carried out to assess whether or not it appeared that fasting status (i.e. whether a subject successfully fasted for a period of 6 hours or not) affected the blood measurements taken. Two way analysis of variance was used to examine the effects of fasting status and diabetic status on each of the measures.

Whether or not a person was a diabetic affected all the measures except fibrinogen. Fasting status was found to significantly affect total cholesterol, LDL cholesterol, triglycerides, insulin and glucose levels, but not HDL cholesterol or fibrinogen. Furthermore, for LDL cholesterol, triglycerides, insulin and glucose, the effect of being a faster rather than a non faster differed significantly in magnitude by diabetics status.

It was therefore decided (Peter & Jon) that an adjustment for fasting durations would be necessary for total cholesterol, LDL cholesterol, triglycerides, insulin and glucose.

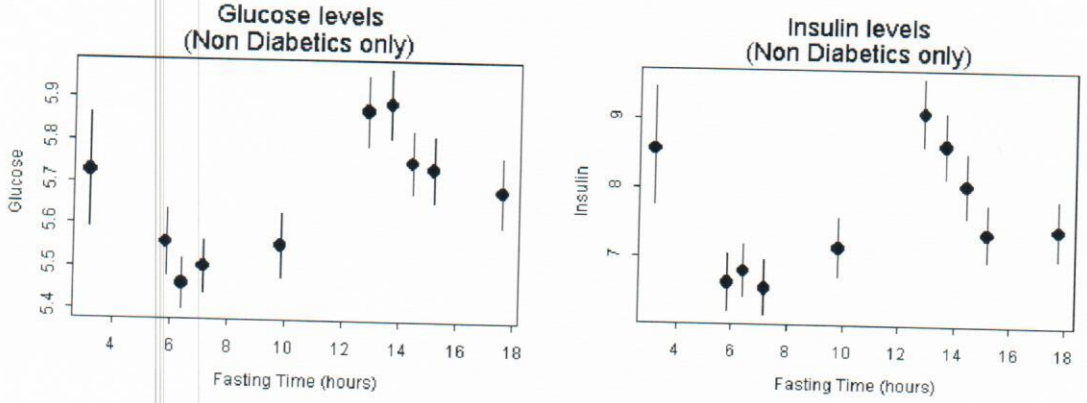
ADJUSTMENT OF TOTAL CHOLESTEROL, LDL CHOLESTEROL AND TRIGLYCERIDES

In fitting models to adjust for fasting status for these variables, it was desirable to keep the models simple.

For total cholesterol, LDL cholesterol and triglycerides, linear regression was used to quantify the differences by diabetic status and fasting status. Since the interaction between diabetic status and fasting status was found to be significant for LDL cholesterol and triglycerides, separate models were fitted for the diabetics and the non diabetics, leaving fasting status as the only explanatory variable. The coefficients from these four analyses were then used to respectively adjust the measures of the non fasters to an amount synonymous with the fasters. For total cholesterol, one model was fitted containing fasting status and diabetic status as the explanatory variables. Non fasters were adjusted to the level of fasters, whilst preserving the differences between diabetics and non diabetics.

INSULIN AND GLUCOSE – A PERIODIC EFFECT?

By examination of insulin and glucose levels for non diabetics, there appeared to be a periodic effect of fasting time where peaks were observed at both the lowest level of fasting, but also for fasting levels around 13 hours. This is clearly indicated below.



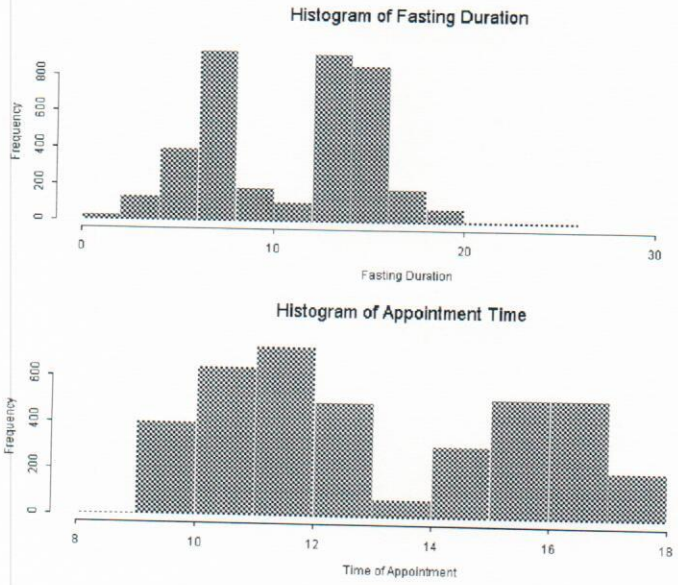
Further examination of the subjects contributing to these data revealed that reported fasting duration was strongly related to time of appointment, with subjects having morning appointments being more likely to report long fasting times (over night) than subjects with afternoon appointments. The second peaks observed for both insulin and glucose levels at 13 hours fasting duration may then be attributable to subjects failing to report accurate fasting times, perhaps they had a drink for breakfast which they failed to report. These peaks may therefore be due to missreporting of fasting times rather than any true periodic effect.

Nevertheless, this needs to be accounted for in an adjustment of these variables[1].

[1] Providing that the adjustment is carried out based on a suitable model derived from the data, it is irrelevant how accurate the fasting times actually are.

TRANSFORMATION OF FASTING DURATION & APPOINTMENT TIME

Both fasting time and time of appointment are bi-modal, as can be seen below. For appointment time, the modes correspond to the peak hours in the morning and afternoon, whilst for fasting duration, the explanation is probably attributable to missclassification. In order to model linearly with these variables certain transformations were carried out.



Fasting time was categorized into one of two intervals, [0,10), or [10, 20)[2], creating a dichotomous variable. The fasting time was also divided modulo 10, creating a continuous variable defined on the half open interval [0, 10). The aim of this was to replace the original data with two variables, a continuous variable and a binary variable[3].

For time of appointment, a similar calculation was performed. All appointments were categorized as either morning or afternoon appointments, where afternoon appointments began at 1 P.M. This binary variable then defines the time of appointment as belonging to one of the intervals [8, 13) or [13, 18), using the 24 hour clock notation. By shifting these intervals to the origin and performing a division modulo 5, we have a continuous variable defined on the half open interval [0, 5)[4].

The purpose of these transformations was to keep all the information contained in the fasting time and time of appointment, whilst facilitating linear modeling of the variables. The transformed continuous variables are shown overleaf, on the square root scale[5].

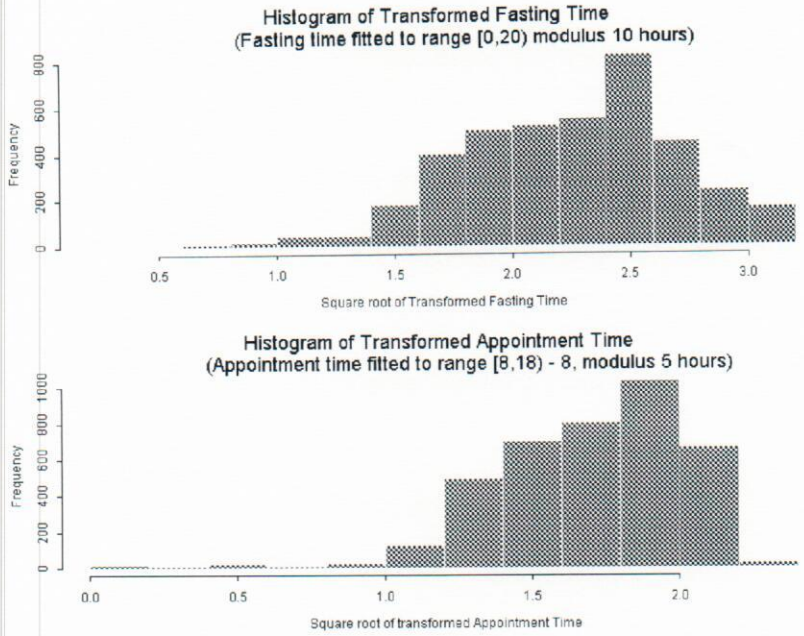
---

[2] Any fasting time that was originally ≥ 20 was changed to 19.99 for the purpose of modulus division.

[3] The new data are just a linear combination of the original data $\xi_{\text{original}} = \xi_{\text{new}} + 10\delta$, where δ equals one or zero, depending on whether ξ_{original} was less than 10 hours.

[4] The new data are just a linear combination of the original data $\tau_{\text{original}} = \tau_{\text{new}} + 8 + 5\gamma$, where γ equals one or zero, depending on whether τ_{original} was less than 13 hours (i.e. before a 1 P.M appointment).

[5] A power transformation method (Box-Cox) could have been used to determine a more suitable power transformation than the square root, but it was decided to keep things as simple as possible from here on.



INSULIN & GLUCOSE ADJUSTMENT

Having transformed the relevant variables, models were fitted for insulin and glucose, for diabetics and non diabetics separately. The main effects of fasting duration (2 independent variables) and time of appointment (2 independent variables) were included along with all 2 way interactions. A backwards elimination procedure was then adopted to select the best model in each case. Based on the coefficients of these models, subjects were then standardised to that of a 6 hour faster with a 1P.M.appointment.

SENSITIVITY ANALYSIS – GLYCOSYLATED HAEMOGLOBIN (HBA1C)

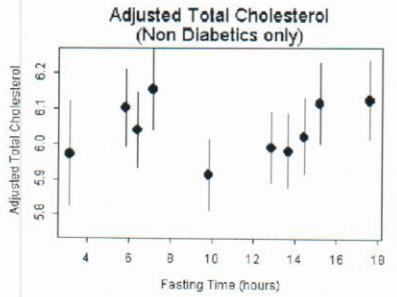
It is possible that the diabetics that failed to fast may in some sense be 'worst case diabetics', and so adjustment of their insulin and glucose levels may actually be adjusting away true differences between the diabetics. To investigate this possibility a sensitivity analysis was carried out for the diabetics that included in the models the variable HBA1C. Backwards elimination of the full model was again carried out for both insulin and glucose.

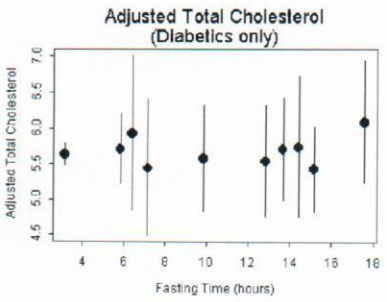
For insulin, the re-fitted model contained the same combination of variables as before (plus HBA1C), and the coefficients were similar in direction and magnitude as in the model where we don't adjust for HBA1C.

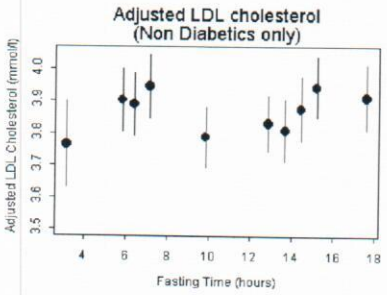
For glucose, the re-fitted model becomes simpler, involving HBA1C and the two main effects of fasting time (transformed continuous variable) and whether or not the appointment was in the morning. HBA1C is included in the adjustment models for the purpose of preserving these differences between diabetics.

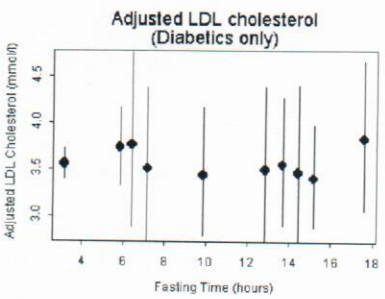
AFTER THE ADJUSTMENT

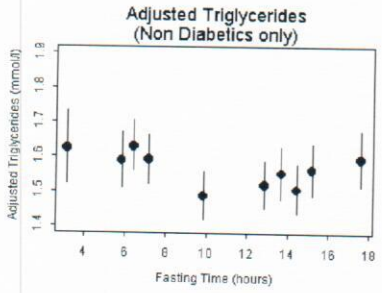
Results of all the adjustments are shown below.

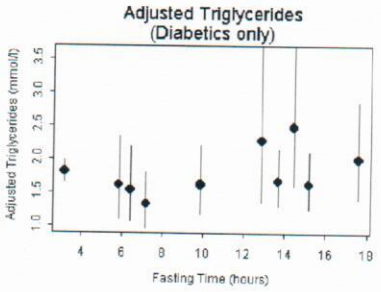


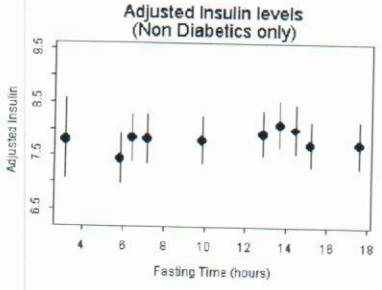


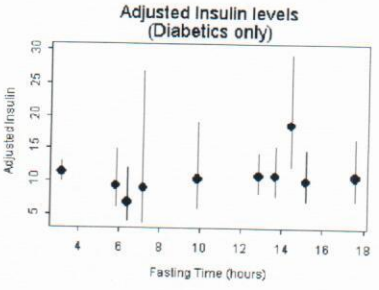


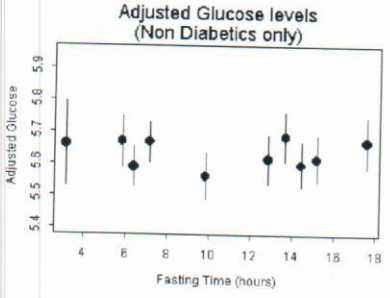


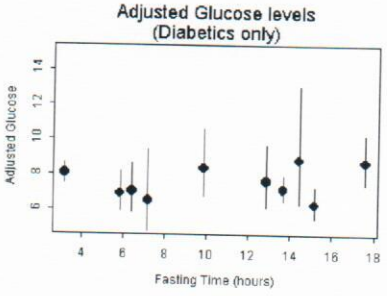












DISCUSSION

For the twenty year follow up of men in the British Regional Heart Study, it was necessary to adjust certain blood measurements to take into account the different fasting times of the subjects. Five measurements were adjusted, total cholesterol, LDL cholesterol, triglycerides, insulin and glucose. Diabetic subjects were adjusted differently to non diabetics for all measures except total cholesterol.

Simple adjustments were carried out for total cholesterol, LDL cholesterol and triglycerides, which only adjusted non fasters (< 6 hours fasting duration) to a level consistent with fasters. This kind of adjustment is straightforward, but makes no distinction between fasting times of non-fasters. Some subjects falling into the category of non fasters would most likely be adjusted by an amount more than that expected if they had truly fasted for 6 hours. Alternatively, some subjects may still have artificially low readings.

For insulin and glucose, it was necessary to make a distinction between afternoon appointments and morning appointments, as it appeared that many subjects may well have miss-reported their true fasting time (potentially due to a drink that they neglected to mention). It is not surprising that such an error would present itself when analysing insulin and glucose, but be hidden from the other measurements. For these two measures then, a more complicated adjustment was performed, including aspects of both fasting time and time of appointment in the adjustment. A sensitivity analysis using the marker of HBA1C yielded similar results in terms of coefficients. HBA1C was included in the final models for insulin and glucose, partly since the model for glucose was simpler with HBA1C in it, than it was without.

No account of town of residence was used in these analyses. It is assumed that fasting status in independent of town of residence.

```
# ============================
# FASTING ADJUSTMENT PROGRAM
# ============================

# In this program we adjust for the fasting duration of each subject.
# For total cholesterol, LDL cholesterol and triglycerides, we adjust for
# fasting status only, i.e. we adjust those who didn't fast to a level
# consistent with those who did (whilst preserving differences between diabetics
# and non diabetics).  No adjustment is carried out for HDL chlesterol as difference
s
# between fasting levels are consistent with a chance event.  For insulin and glucos
e
# we adjust based on two factors, fasting duration and time of appointment, both of
# which we consider as continuous variables.  We standardise responses to those of a

# 6 hour faster with a 1pm appointment (the mean appointment time).


import.data(FileName = "C:\\work\\brhs\\fasting adjustment\\output\\Fstm_tod.sd2",
        FileType = "SAS",
        ColNames = "",
        Format = "",
        TargetStartCol = "1",
        DataFrame = "fasting.adjustment.data",
        NameRow = "",
        StartCol = "1",
        EndCol = "END",
        StartRow = "1",
        EndRow = "END",
        Delimiters = ", \t",
        SeparateDelimiters = F,
        PageNumber = "1",
        RowNameCol = "",
        StringsAsFactors = "Auto",
        VLabelAsNumber = F,
        Filter = "",
        OdbcConnection = "",
        OdbcSqlQuery = "")

  fasting.adjustment.data$DIABETES <- factor(fasting.adjustment.data$DIABETES)
  fasting.adjustment.data$DECGRP <- factor(fasting.adjustment.data$DECGRP)

##################################################
########  TOTAL CHOLESTEROL ADJUSTMENT ###########
##################################################

#  WE FOUND THAT FOR TOTAL CHOLESTEROL, NON FASTERS (< 6 HRS) HAD ON AVERAGE
#  LOWER CHOLESTEROL LEVELS THAN FASTERS (>6 HRS), AND THAT DIABETICS
#  HAD LOWER CHOLESTEROL LEVELS THAN NON-DIABETICS.  THE DIFFERENCE BETWEEN
#  FASTERS AND NON-FASTERS DID NOT VARY BY DIABETIC STATUS (NON-SIGNIFICANT
#  INTERACTION)

        chol.model <- lm(CHOL ~ DIABETES + SUC.FAST, data=fasting.adjustment.data, n
a.action=na.omit)
        fasting.adjustment.data$CHOL <- fasting.adjustment.data$CHOL +
                                (fasting.adjustment.data$SUC.FAST==0)*coef(chol.mode
l)[3]

##################################################
########  HDL CHOLESTEROL ADJUSTMENT ###########
##################################################

# NO ADJUSTMENT IS CARRIED OUT FOR HDL CHOLESTEROL.  AS WE CAN SEE FROM THE
# FOLLOWING REGRESSION, NO ADJUSTMENT IS NECESSARY.

        hdl.model <- lm(sqrt(HDL) ~ DIABETES + SUC.FAST, data=fasting.adjustment.dat
a, na.action=na.omit)
        summary(hdl.model)
```

```
################################################
########  LDL CHOLESTEROL ADJUSTMENT ############
################################################

        ## THE ORIGINAL DATA SHOW SIGNIFICANT MAIN EFFECTS DUE TO DIABETIC STATUS AN
D FASTING STATUS
        ## THEY ALSO INDICATE A SIGNIFICANT INTERACTION WHICH IS EVIDENT FROM THE IN
TERACTION PLOTS
        ## CONTAINED IN 'C:\WORK\BRHS\FASTING ADJUSTMENT\GRAPHS\INTERACTION PLOTS 1
& 2'

        ## WE THERFORE ADJUST THE DIABETICS AND NON DIABETICS SEPARATELY BASED ON 2
MODELS

        # STEP 1- ADJUST THE NON-DIABETICS
        # ================================
        ldl.model <- lm(sqrt(LDL) ~ SUC.FAST, data=fasting.adjustment.data, na.actio
n=na.omit,
                                        subset=(fasting.adjustment.data$DIABETES!=1)
)
        summary(ldl.model)
        new.sqrt.ldl <- sqrt(fasting.adjustment.data$LDL[fasting.adjustment.data$DIA
BETES!=1]) +
                                (fasting.adjustment.data$SUC.FAST[fasting.adjustment
.data$DIABETES!=1]==0)*coef(ldl.model)[2]
        fasting.adjustment.data$LDL[fasting.adjustment.data$DIABETES!=1] <- new.sqrt
.ldl^2


        # STEP 2 - ADJUST THE DIABETICS
        # =====================================
        ldl.model3 <- lm(sqrt(LDL) ~ SUC.FAST, data=fasting.adjustment.data, na.acti
on=na.omit,
                                        subset=(fasting.adjustment.data$DIABETES==1)
)
        summary(ldl.model3)
        new.sqrt.ldl <- sqrt(fasting.adjustment.data$LDL[fasting.adjustment.data$DIA
BETES==1]) +
                                (fasting.adjustment.data$SUC.FAST[fasting.adjustment
.data$DIABETES==1]==0)*coef(ldl.model3)[2]
        fasting.adjustment.data$LDL[fasting.adjustment.data$DIABETES==1] <- new.sqrt
.ldl^2



################################################
########  TRIGLYCERIDES ADJUSTMENT ############
################################################

        # SIMILAR SITUATION TO LDL CHOLESTEROL -> ADJUST USING TWO MODELS

        # STEP 1- ADJUST THE NON-DIABETICS
        # ================================
        trig.model <- lm(log(TRIGS) ~ SUC.FAST, data=fasting.adjustment.data,
                                        na.action=na.omit, subset=(fasting.adjustmen
t.data$DIABETES!=1))
        summary(trig.model)
        new.logtrig <- log(fasting.adjustment.data$TRIGS[fasting.adjustment.data$DIA
BETES!=1]) +
                                (fasting.adjustment.data$SUC.FAST[fasting.adjustment
.data$DIABETES!=1]==0)*coef(trig.model)[2]
        fasting.adjustment.data$TRIGS[fasting.adjustment.data$DIABETES!=1] <- exp(ne
w.logtrig)


        # STEP 2 - ADJUST THE DIABETICS
        # =================================
        trig.model3 <- lm(log(TRIGS) ~ SUC.FAST, data=fasting.adjustment.data, na.ac
tion=na.omit,
```

```
                                                subset=(fasting.adjustment.data$DIABETES==1)
 )
        summary(trig.model3)
        new.logtrig <- log(fasting.adjustment.data$TRIGS[fasting.adjustment.data$DIA
 BETES==1]) +
                                    (fasting.adjustment.data$SUC.FAST[fasting.adjustment
 .data$DIABETES==1]==0)*coef(trig.model3)[2]
        fasting.adjustment.data$TRIGS[fasting.adjustment.data$DIABETES==1] <- exp(ne
 w.logtrig)



 ##########################
 ### INSULIN ADJUSTMENT ###
 ##########################

 # Both the fasting time and time of appointment are bi-modal
 # Fasting time reaches a peak at 7 hours, falls towards 10 hours, and then
 # reaches another peak at approximately 15 hours. NB/ 55% of the subjects fasted
 # for at least 10 hours.
 # Time of appointment has two peaks corresponding to the busy periods
 # in the morning and the afternoon.
 # We transform these variables to be normal in the following way:
 # For fasting time we take as the new variable fasting time modulus 10 (NB/ We
 # don't lose the information on who fasted as we keep the variable LONGFAST, defined
  to be 1
 # if the subject fasted for >= 10 hours, and a zero otherwise)
 # For time of appointment we take as the new variable the number of hours since
 # either 8am or 1pm, depending on whether the appointment is in the afternoon or mor
 ning.
 # NB/ We don't lose the information regarding the morning/afternoon appointments, si
 nce
 # we keep the binary varible MORNING.

 par(mfrow=c(2,1))
 hist(fasting.adjustment.data$FAST.TM[fasting.adjustment.data$DIABETES!=1], xlab='Fas
 ting Duration', ylab='Frequency', main='Histogram of Fasting Duration')
 hist(fasting.adjustment.data$TIMEDSX[fasting.adjustment.data$DIABETES!=1], xlab='Tim
 e of Appointment', ylab='Frequency', main='Histogram of Appointment Time')

 past.12hours <- fasting.adjustment.data$FAST.TM >= 20
 fasting.adjustment.data$FAST.TM[past.12hours] <- 19.99
 fasting.adjustment.data$FAST.TM <- fasting.adjustment.data$FAST.TM %% 10
 past.6pm <- fasting.adjustment.data$TIMEDSX >= 18
 fasting.adjustment.data$TIMEDSX[past.6pm] <- 17.99
 fasting.adjustment.data$TIMEDSX <- (fasting.adjustment.data$TIMEDSX - 8) %% 5

 hist(sqrt(fasting.adjustment.data$FAST.TM[fasting.adjustment.data$DIABETES!=1]), xla
 b='Square root of Transformed Fasting Time', ylab='Frequency', main='Histogram of Tr
 ansformed Fasting Time
 (Fasting time fitted to range [0,20) modulus 10 hours)')
 hist(sqrt(fasting.adjustment.data$TIMEDSX[fasting.adjustment.data$DIABETES!=1]), xla
 b='Square root of transformed Appointment Time', ylab='Frequency', main='Histogram o
 f Transformed Appointment Time
 (Appointment time fitted to range [8,18) - 8, modulus 5 hours)')

        # STEP 1- ADJUSTMENT FOR TIME OF APPOINTMENT AND FASTING STATUS - FOR THE NO
 N DIABETICS ONLY
        # ============================================================================
 ================
        ins.model1 <- lm(log(INSULIN) ~ LONGFAST + MORNING + sqrt(FAST.TM) + sqrt(TI
 MEDSX) +        MORNING:sqrt(TIMEDSX)  + LONGFAST:sqrt(FAST.TM)

                                                ,data=fasting.adjustment.data, na.ac
 tion=na.omit,
                                                subset=(fasting.adjustment.data$DIAB
 ETES!=1))
        summary(ins.model1)
```

```
        # ADJUST THE NON-DIABETIC INSULIN LEVELS TO STANDARDISE TO A 6 HOUR FASTER W
ITH A 1PM APPOINTMENT
        # i.e.   LONGFAST=0, MORNING=0, FAST.TM=6, TIMEDSX=0
        new.logins <- log(fasting.adjustment.data$INSULIN[fasting.adjustment.data$DI
ABETES!=1]) +
                                        (0-fasting.adjustment.data$LONGFAST[fasting.
adjustment.data$DIABETES!=1])*coef(ins.model1)[2] +
                                        (0-fasting.adjustment.data$MORNING[fasting.a
djustment.data$DIABETES!=1])*coef(ins.model1)[3] +
                                        (sqrt(6)-sqrt(fasting.adjustment.data$FAST.T
M[fasting.adjustment.data$DIABETES!=1]))*coef(ins.model1)[4] +
                                        (0-sqrt(fasting.adjustment.data$TIMEDSX[fast
ing.adjustment.data$DIABETES!=1]))*coef(ins.model1)[5] +
                                    (0-(fasting.adjustment.data$MORNING*sqrt(fasting.adj
ustment.data$TIMEDSX))[fasting.adjustment.data$DIABETES!=1])*coef(ins.model1)[6] +
                                    (0-(fasting.adjustment.data$LONGFAST*sqrt(fasting.ad
justment.data$FAST.TM))[fasting.adjustment.data$DIABETES!=1])*coef(ins.model1)[7]

        fasting.adjustment.data$INSULIN[fasting.adjustment.data$DIABETES!=1] <- exp(
new.logins)



        # STEP 2 - ADJUSTMENT FOR TIME OF APPOINTMENT AND FASTING STATUS - FOR THE D
IABETICS ONLY
        # ==========================================================================
=============

        # The non-fasting diabetics may differ from the fasting diabetics as 'worst
case' diabetics.
        # To account for this possibility, we use models adjusting for HBA1C of the
diabetics.
        # We do this for Insulin and also for glucose

        hist(fasting.adjustment.data$HBA1C[fasting.adjustment.data$DIABETES==1], mai
n='HBA1C
        Diabetics only', xlab='HBA1C', ylab='Frequency')
        hist(log(fasting.adjustment.data$HBA1C[fasting.adjustment.data$DIABETES==1])
, main='HBA1C (Log Scale)
        Diabetics only', xlab='HBA1C (Log Scale)', ylab='Frequency')


        ins.model3 <- lm(log(INSULIN) ~ log(HBA1C) + LONGFAST + MORNING + sqrt(FAST.
TM)+ LONGFAST:MORNING + MORNING:sqrt(FAST.TM)
                                                , data=fasting.adjustment.data, na.a
ction=na.omit,
                                                subset=(fasting.adjustment.data$DIAB
ETES==1))
        summary(ins.model3)

        # ADJUST THE NON-DIABETIC INSULIN LEVELS TO STANDARDISE TO A 6 HOUR FASTER W
ITH A 1PM APPOINTMENT
        # i.e.   LONGFAST=0, MORNING=0, FAST.TM=6, TIMEDSX=0
        # THE MEAN FASTING TIME FOR THE DIABETICS WAS 6.3 HOURS

        new.logins <- log(fasting.adjustment.data$INSULIN[fasting.adjustment.data$DI
ABETES==1]) +
                                        (0-fasting.adjustment.data$LONGFAST[fasting.
adjustment.data$DIABETES==1])*coef(ins.model3)[3] +
                                        (0-fasting.adjustment.data$MORNING[fasting.a
djustment.data$DIABETES==1])*coef(ins.model3)[4] +
                                        (sqrt(6)-sqrt(fasting.adjustment.data$FAST.T
M[fasting.adjustment.data$DIABETES==1]))*coef(ins.model3)[5] +
                                    (0-(fasting.adjustment.data$MORNING*fasting.adjustme
nt.data$LONGFAST)[fasting.adjustment.data$DIABETES==1])*coef(ins.model3)[6] +
                                    (0-(fasting.adjustment.data$MORNING*sqrt(fasting.adj
ustment.data$FAST.TM))[fasting.adjustment.data$DIABETES==1])*coef(ins.model3)[7]

        fasting.adjustment.data$INSULIN[fasting.adjustment.data$DIABETES==1] <- exp(
```

```
new.logins)



##########################
### GLUCOSE ADJUSTMENT ###
##########################


        # STEP 1- ADJUSTMENT FOR TIME OF APPOINTMENT AND FASTING STATUS - FOR THE NO
N DIABETICS ONLY
        # ================================================================================
================

        glu.model1 <- lm(log(log(GLU)) ~ LONGFAST + MORNING + sqrt(FAST.TM) + sqrt(T
IMEDSX) + MORNING:sqrt(FAST.TM)+MORNING:sqrt(TIMEDSX)
                                                ,data=fasting.adjustment.data, na.ac

tion=na.omit,
                                                subset=(fasting.adjustment.data$DIAB

ETES!=1))
        summary(glu.model1)

        # ADJUST THE NON-DIABETIC GLUOSE LEVELS TO STANDARDISE TO A 6 HOUR FASTER WI
TH A 1PM APPOINTMENT
        # i.e.   LONGFAST=0, MORNING=0, FAST.TM=6, TIMEDSX=0
        new.glu <- log(log(fasting.adjustment.data$GLU[fasting.adjustment.data$DIABE
TES!=1])) +
                                        (0-fasting.adjustment.data$LONGFAST[fasting.

adjustment.data$DIABETES!=1])*coef(glu.model1)[2] +
                                        (0-fasting.adjustment.data$MORNING[fasting.a

djustment.data$DIABETES!=1])*coef(glu.model1)[3] +
                                        (sqrt(6)-sqrt(fasting.adjustment.data$FAST.T

M[fasting.adjustment.data$DIABETES!=1]))*coef(glu.model1)[4] +
                                        (0-sqrt(fasting.adjustment.data$TIMEDSX[fast

ing.adjustment.data$DIABETES!=1]))*coef(glu.model1)[5] +
                                (0-(fasting.adjustment.data$MORNING*sqrt(fasting.adj

ustment.data$FAST.TM))[fasting.adjustment.data$DIABETES!=1])*coef(glu.model1)[6] +
                                (0-(fasting.adjustment.data$MORNING*sqrt(fasting.adj

ustment.data$TIMEDSX))[fasting.adjustment.data$DIABETES!=1])*coef(glu.model1)[7]

        fasting.adjustment.data$GLU[fasting.adjustment.data$DIABETES!=1] <- exp(exp(

new.glu))


        # STEP 2 - ADJUSTMENT FOR TIME OF APPOINTMENT AND FASTING STATUS - FOR THE D
IABETICS ONLY
        # ================================================================================
=============

        glu.model3 <- lm(log(log(GLU)) ~ log(HBA1C) + MORNING + sqrt(FAST.TM)
                                                ,data=fasting.adjustment.data, na.action=na.

omit,
                                                subset=(fasting.adjustment.data$DIAB

ETES==1))
        summary(glu.model3)

        # ADJUST THE DIABETIC GLUOSE LEVELS TO STANDARDISE TO A 6 HOUR FASTER WITH A
 1PM APPOINTMENT
        # i.e.   MORNING=0, FAST.TM=6
        new.glu <- log(log(fasting.adjustment.data$GLU[fasting.adjustment.data$DIABE
TES==1])) +
                                        (0-fasting.adjustment.data$MORNING[fasting.a

djustment.data$DIABETES==1])*coef(glu.model3)[3] +
                                        (sqrt(6)-sqrt(fasting.adjustment.data$FAST.T

M[fasting.adjustment.data$DIABETES==1]))*coef(glu.model3)[4]

        fasting.adjustment.data$GLU[fasting.adjustment.data$DIABETES==1] <- exp(exp(

new.glu))
```

```
#### CHECK THAT ALL THE ADJUSTMENTS HAVE DONE THEIR JOB !

# TOTAL CHOLESTEROL
        chol.model <- lm(CHOL ~ DIABETES + SUC.FAST, data=fasting.adjustment.data, n
a.action=na.omit)
        summary(chol.model)

# LDL CHOLESTEROL
        ldl.model2 <- lm(sqrt(LDL) ~ SUC.FAST, data=fasting.adjustment.data, na.acti
on=na.omit,
                                        subset=(fasting.adjustment.data$DIABETES!=1)
)
        summary(ldl.model2)
        ldl.model4 <- lm(sqrt(LDL) ~ SUC.FAST, data=fasting.adjustment.data, na.acti
on=na.omit,
                                        subset=(fasting.adjustment.data$DIABETES==1)
)
        summary(ldl.model4)

# TRIGLYCERIDES
        trig.model2 <- lm(log(TRIGS) ~ SUC.FAST, data=fasting.adjustment.data,
                                        na.action=na.omit, subset=(fasting.adjustmen
t.data$DIABETES!=1))
        summary(trig.model2)
        trig.model4 <- lm(log(TRIGS) ~ SUC.FAST, data=fasting.adjustment.data, na.ac
tion=na.omit,
                                        subset=(fasting.adjustment.data$DIABETES==1)
)
        summary(trig.model4)

# INSULIN
        ins.model2 <- lm(log(INSULIN) ~ LONGFAST + MORNING + sqrt(FAST.TM) + sqrt(TI
MEDSX) +        MORNING:sqrt(TIMEDSX)  + LONGFAST:sqrt(FAST.TM)
                                                , data=fasting.adjustment.data, na.a
ction=na.omit,
                                                subset=(fasting.adjustment.data$DIAB
ETES!=1))
        summary(ins.model2)
        ins.model4 <- lm(log(INSULIN) ~ log(HBA1C) + LONGFAST + MORNING + sqrt(FAST.
TM) + LONGFAST:MORNING + MORNING:sqrt(FAST.TM)
                                                , data=fasting.adjustment.data, na.a
ction=na.omit,
                                                subset=(fasting.adjustment.data$DIAB
ETES==1))
        summary(ins.model4)

# GLUCOSE
        glu.model2 <- lm(log(log(GLU)) ~ LONGFAST + MORNING + sqrt(FAST.TM) + sqrt(T
IMEDSX) + MORNING:sqrt(FAST.TM)+MORNING:sqrt(TIMEDSX)
                                                ,data=fasting.adjustment.data, na.ac
tion=na.omit,
                                                subset=(fasting.adjustment.data$DIAB
ETES!=1))
        summary(glu.model2)
        glu.model4 <- lm(log(log(GLU)) ~ log(HBA1C)+ MORNING + sqrt(FAST.TM)
                                        ,data=fasting.adjustment.data, na.action=na.
omit,
                                                subset=(fasting.adjustment.data$DIAB
ETES==1))
        summary(glu.model4)


ADJUSTED.DATA <- data.frame(SERIAL=fasting.adjustment.data$SERIAL,
                                                                NEWCHOL=fasting.adj
ustment.data$CHOL,
                                                                NEWLDL=fasting.adju
```

```
stment.data$LDL,
                                                        NEWTRIG=fasting.adj
ustment.data$TRIGS,
                                                        NEWINS=fasting.adju
stment.data$INSULIN,
                                                        NEWGLU=fasting.adju
stment.data$GLU
                                                       )

export.data(DataSet = "ADJUSTED.DATA",
        Columns = "ALL",
        Rows = "ALL",
        Delimiter = ",",
        ColumnNames = T,
        RowNames = T,
        Quotes = T,
        LineLength = "",
        FileName = "C:\\work\\brhs\\data\\data2000\\FAST_ADJ.sd2",
        FileType = "SAS",
        OdbcConnection = "",
        OdbcTable = "",
        FormatString = "")
```

```
        run;


*============================*;
*= Median of decile groups =*;
*============================*;
proc sort; by decgrp; run;
proc univariate data=fast noprint;
        var fast_tm;
        by decgrp;
        where decgrp ne .;
        output out=meds median=medtime;
        run;


*================*;
*= Non Diabetic =*;
*================*;
proc univariate data=fast noprint;
        var chol sqthdl sqtldl logtrigs logins sqtfib logglu sqhba1c;
        by decgrp;
        where decgrp ne . and diabetes ne 1;
        output out=deciles mean = chol sqthdl sqtldl logtrigs logins sqtfib logglu
sqHBA1C
                                  stdmean = sechol sehdl seldl setrigs seinsul sefib seglu
 seHBA1C
                                  n = nchol nhdl nldl ntrigs ninsulin nfib nglu  nHBA1C;
        run;

data nondiab;
        merge meds deciles;
        by decgrp;
        chol=chol;
        hdl=sqthdl ** 2;
        ldl=sqtldl ** 2;
        trigs=exp(logtrigs);
        insulin=exp(logins2);
        fib=sqtfib ** 2;
        glu=exp(exp(logglu));
        HBA1C=sqHBA1C ** 2;

        up_chol = chol + (tinv(0.975, nchol-1)*sechol);
        up_hdl = (sqthdl + (tinv(0.975, nhdl-1)*sehdl)) ** 2;
        up_ldl = (sqtldl + (tinv(0.975, nldl-1)*seldl)) ** 2;
        up_trigs = exp(logtrigs + (tinv(0.975, ntrigs-1)*setrigs));
        up_ins = exp((logins + (tinv(0.975, ninsulin-1)*seinsul)));
        up_fib = (sqtfib + (tinv(0.975, nfib-1)*sefib)) ** 2;
        up_glu = exp(exp(logglu + (tinv(0.975, nglu-1)*seglu)));
        up_HBA1C = (sqHBA1C + (tinv(0.975, nHBA1C-1)*seHBA1C)) ** 2;

        lo_chol = chol - (tinv(0.975, nchol-1)*sechol);
        lo_hdl = (sqthdl - (tinv(0.975, nhdl-1)*sehdl)) ** 2;
        lo_ldl = (sqtldl - (tinv(0.975, nldl-1)*seldl)) ** 2;
        lo_trigs = exp(logtrigs - (tinv(0.975, ntrigs-1)*setrigs));
        lo_ins = exp((logins - (tinv(0.975, ninsulin-1)*seinsul)));
        lo_fib = (sqtfib - (tinv(0.975, nfib-1)*sefib)) ** 2;
        lo_glu = exp(exp(logglu - (tinv(0.975, nglu-1)*seglu)));
        lo_HBA1C = (sqHBA1C - (tinv(0.975, nHBA1C-1)*seHBA1C)) ** 2;

        keep medtime chol hdl ldl trigs insulin fib glu HBA1C up_chol up_hdl up_ldl
up_trigs
             up_ins up_fib up_glu up_HBA1C lo_chol lo_hdl lo_ldl lo_trigs lo_ins lo_
fib lo_glu lo_HBA1C;
        run;


*=============*;
*= Diabetic =*;
*=============*;
```

```
proc univariate data=fast noprint;
        var chol sqthdl sqtldl logtrigs logins sqtfib logglu sqHBA1C;
        by decgrp;
        where decgrp ne . and diabetes=1;
        output out=deciles mean = chol sqthdl sqtldl logtrigs logins sqtfib logglu s
qHBA1C
                           stdmean = sechol sehdl seldl setrigs seinsul sefib seglu
seHBA1C
                           n = nchol nhdl nldl ntrigs ninsulin nfib nglu nHBA1C;
        run;
data diabetic;
        merge meds deciles;
        by decgrp;
        chol=chol;
        hdl=sqthdl ** 2;
        ldl=sqtldl ** 2;
        trigs=exp(logtrigs);
        insulin=exp(logins);
        fib=sqtfib ** 2;
        glu=exp(exp(logglu));
        HBA1C=sqHBA1C ** 2;

        up_chol = chol + (tinv(0.975, nchol-1)*sechol);
        up_hdl = (sqthdl + (tinv(0.975, nhdl-1)*sehdl)) ** 2;
        up_ldl = (sqtldl + (tinv(0.975, nldl-1)*seldl)) ** 2;
        up_trigs = exp(logtrigs + (tinv(0.975, ntrigs-1)*setrigs));
        up_ins = exp((logins + (tinv(0.975, ninsulin-1)*seinsul)));
        up_fib = (sqtfib + (tinv(0.975, nfib-1)*sefib)) ** 2;
        up_glu = exp(exp(logglu + (tinv(0.975, nglu-1)*seglu)));
        up_HBA1C = (sqHBA1C + (tinv(0.975, nHBA1C-1)*seHBA1C)) ** 2;

        lo_chol = chol - (tinv(0.975, nchol-1)*sechol);
        lo_hdl = (sqthdl - (tinv(0.975, nhdl-1)*sehdl)) ** 2;
        lo_ldl = (sqtldl - (tinv(0.975, nldl-1)*seldl)) ** 2;
        lo_trigs = exp(logtrigs - (tinv(0.975, ntrigs-1)*setrigs));
        lo_ins = exp((logins - (tinv(0.975, ninsulin-1)*seinsul)));
        lo_fib = (sqtfib - (tinv(0.975, nfib-1)*sefib)) ** 2;
        lo_glu = exp(exp(logglu - (tinv(0.975, nglu-1)*seglu)));
        lo_HBA1C = (sqHBA1C - (tinv(0.975, nHBA1C-1)*seHBA1C)) ** 2;

        keep medtime chol hdl ldl trigs insulin fib glu HBA1C up_chol up_hdl up_ldl
up_trigs
             up_ins up_fib up_glu up_HBA1C lo_chol lo_hdl lo_ldl lo_trigs lo_ins lo_
fib lo_glu lo_HBA1C;

        run;


*============================================*;
*= Write these data sets to external files =*;
*============================================*;

%macro write(data, mean, lower, upper, out);
        data &out;
                set &data;
                mean=&mean;
                lower=&lower;
                upper=&upper;
                keep medtime mean lower upper;
                run;
%mend;

%write(nondiab, chol, lo_chol, up_chol, lib1.chol_ND);
%write(nondiab, hdl, lo_hdl, up_hdl, lib1.hdl_ND);
%write(nondiab, ldl, lo_ldl, up_ldl, lib1.ldl_ND);
%write(nondiab, trigs, lo_trigs, up_trigs, lib1.trigs_ND);
%write(nondiab, insulin, lo_ins, up_ins, lib1.ins_ND);
%write(nondiab, fib, lo_fib, up_fib, lib1.fib_ND);
%write(nondiab, glu, lo_glu, up_glu, lib1.glu_ND);
%write(nondiab, HBA1C, lo_HBA1C, up_HBA1C, lib1.HBA1C_ND);
```

```
%write(diabetic, chol, lo_chol, up_chol, lib1.chol_DB);
%write(diabetic, hdl, lo_hdl, up_hdl, lib1.hdl_DB);
%write(diabetic, ldl, lo_ldl, up_ldl, lib1.ldl_DB);
%write(diabetic, trigs, lo_trigs, up_trigs, lib1.trigs_DB);
%write(diabetic, insulin, lo_ins, up_ins, lib1.ins_DB);
%write(diabetic, fib, lo_fib, up_fib, lib1.fib_DB);
%write(diabetic, glu, lo_glu, up_glu, lib1.glu_DB);
%write(diabetic, HBA1C, lo_HBA1C, up_HBA1C, lib1.HBA1C_DB);


*=======================================*;
*=  Produce data for factor level plots =*;
*=======================================*;
proc sort data=fast; by suc_fast diabetes; run;
proc univariate data=fast noprint;
        var chol hdl ldl trigs insulin fib glu;
        by suc_fast diabetes;
        where diabetes ne . and suc_fast ne .;
        output out=fac mean= chol hdl ldl trigs insulin fib glu;
        run;



*=============================================================*;
*= For the non-diabetics, explore the relationship between  =*;
*= fasting decile group and time of appointment             =*;
*=============================================================*;

proc sort data=fast; by decgrp; run;
proc univariate data=fast plot;
        var timedsx;
        by decgrp;
        where diabetes ne 1;
        run;



data lib1.factplot;
        set fac;
        run;
data lib1.fstm_tod;
        set fast;
        if diabetes=. then diabetes=2;
        if fast_tm ne .;
        if timedsx < 13 then morning=1; else morning=0;
        if fast_tm >= 10 then longfast=1; else longfast=0;
        keep SERIAL timedsx fast_tm decgrp diabetes insulin glu morning chol ldl tri
gs suc_fast hdl
             longfast hba1c;
        run;
```

```
*-------------------------------------------------------------------------*;
*-  The information gained from the blood samples may depend on          -*;
*-  whether or not the subject fasted for the requested 6 hours          -*;
*-  before examination.                                                  -*;
*-                                                                       -*;
*-  We determine here which variables are affected by fasting status     -*;
*-  (note the role of diabetic status and work out what to do about it   -*;
*-------------------------------------------------------------------------*;


libname lib1 'c:\work\brhs\fasting adjustment\output';

options nodate nonumber;

%inc 'c:\work\brhs\sasprg\formatting.sas';
%inc 'c:\work\brhs\sasprg\read in baseline data\towns.sas';
%inc 'c:\work\brhs\sasprg\read in baseline data\ages.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\blue questionnaire.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\data sheets.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\biochemical data.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\haematological data.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\plasma.sas';
%inc 'c:\work\brhs\sasprg\read in 2000 data\insulin.sas';


data fast (rename=(n51=diabetes));
        merge bluall (in=flag1) alldsx towns ages allhea allbio plasma insulin;
        by serial;
        if flag1;

        *-- Height --*;
        if readinad ne . then height=.;

        *-- BMI --*;
        BMI = (10000 * actweigh) / (height * height);

        *---  Sort out the fasting times --*;
        timedsx = time + (time1/60);
        if timedsx=. then timedsx=time;
        last_ate = n200h + (n200m/60);
        if last_ate=. then last_ate=n200h;

        *-- After checking the blue questionnaire we change the following --*;
        *=======================================*;
        if serial=241534 then timedsx=16 + (50/60);
        *=======================================*;
        if (last_ate>timedsx and last_ate ne . and timedsx ne .)
                then fast_tm = timedsx + (24-last_ate);
        if (last_ate<=timedsx and last_ate ne . and timedsx ne .)
                then fast_tm = timedsx - last_ate;

        *-- Andy and myself agreed to set time of the last meal to 9pm --*;
        *-- if they ate yesterday but gave no time                     --*;
        *-- An ASCII file of serial number and fasting time was        --*;
        *-- produced and circulated to everyone in the BRHS            --*;

        if n200Y=1 and last_ate=. then fast_tm=timedsx+3;
        if n200y=1 and (last_ate<=timedsx and last_ate ne . and timedsx ne .)
                then fast_tm = 24 + timedsx - last_ate;
        if last_ate=0 and n200y=1 then fast_tm=timedsx-last_ate;

        if fast_tm >=6 then suc_fast=1; else suc_fast=0;
        if fast_tm=. then suc_fast=.;

        format town town. n51 yesnof.;

run;

proc sort; by serial; run;
```

```
data _null_;
        set fast;
        file 'c:\work\brhs\data\data2000\fastingtimes.dat';
        put @ 1 serial @ 20 fast_tm;
        run;



*=========================;
*=  fasting by diabetes =*;
*=========================;

proc freq data=fast;
        tables suc_fast;
        where suc_fast ne .;
        run;
proc freq data=fast;
        tables diabetes;
        where diabetes ne .;
        run;
proc freq data=fast;
        tables suc_fast * diabetes / nopercent;
        where suc_fast ne . and diabetes ne .;
        run;

*=============================================================================*;
*=  Is there a fasting  / non-fasting difference re. blood variables? =*;
*=============================================================================*;

data fast;
        set fast;
        sqtfib = sqrt(fib);
        sqthdl = sqrt(hdl);
        sqtldl = sqrt(ldl);
        logins = log(insulin);
        logtrigs = log(trigs);
        logglu=log(log(glu));
        sqhba1c=sqrt(hba1c);
        label sqtfib='Square root of fibrinogen'
              sqthdl='Square root of HDL cholesterol'
              sqtldl='Square root of LDL cholesterol'
              logins='Log insulin'
              logtrigs='Log of triglycerides'
              logglu='Log Glucose'
              ;
        run;

*=============================================*;
*-- Do a Friedman Test in S-Plus for glucose --*;
*=============================================*;
data lib1.friedman;
        set fast;
        if suc_fast ne . and diabetes ne .;
        keep glu suc_fast diabetes;
        run;


*============================*;
*-- Do ANOVAs for the rest --*;
*============================*;
%macro doanova1(var, title);
 proc glm data=fast;
       title1 '2 way ANOVA - '  &title;
       title2 'Null hyp is that fasting has no effect';
       class suc_fast diabetes;
       model &var=suc_fast diabetes suc_fast*diabetes;
       means suc_fast diabetes;
       run;
%mend;
```

```
%macro doanova2(var, title);
 proc glm data=fast;
       title1 '2 way ANOVA - '  &title;
       title2 'Null hyp is that fasting has no effect';
       class suc_fast diabetes;
       model &var=suc_fast diabetes;
       means suc_fast diabetes;
       run;
%mend;


%doanova2(chol, 'Total Cholesterol');
%doanova2(sqthdl, 'HDL Cholesterol');
%doanova1(sqtldl, 'LDL cholesterol');
%doanova1(logtrigs, 'Triglycerides');
%doanova1(sqtfib, 'Fibrinogen');

 proc glm data=fast;
       title1 '2 way ANOVA - Fibrinogen (Non Diabetics)';
       title2 'Null hyp is that fasting has no effect';
       class suc_fast;
       model sqtfib=suc_fast;
       means suc_fast;
       where diabetes ne 1;
       run;
 proc glm data=fast;
       title1 '2 way ANOVA - Fibrinogen (Diabetics)';
       title2 'Null hyp is that fasting has no effect';
       class suc_fast;
       model sqtfib=suc_fast;
       means suc_fast;
       where diabetes = 1;
       run;

%doanova1(logins, 'Insulin');
%doanova1(logglu, 'Glucose');


*================================================================;
*================================================================;

* THEN SPLIT FASTING TIMES INTO DECILES AND CATEGORIZE SUBJECTS ;
* AND LOOK AT MEAN (95% CI) LEVELS OF EACH VARIABLE --> SEARCH  ;
* FOR A POSSIBLE THRESHOLD RELATIONSHIP !!!!!                   ;

*================================================================;
*================================================================;


*=========================*;
*= Classify into deciles =*;
*=========================*;
proc univariate data=fast noprint;
       var fast_tm;
       output out=deciles pctlpre=p pctlpts=10 20 30 40 50 60 70 80 90;
       run;

data fast;
       set fast;
       if .<fast_tm<=4.98333 then decgrp=1;
       if 4.98333<fast_tm<=6.15 then decgrp=2;
       if 6.15<fast_tm<=6.725 then decgrp=3;
       if 6.725<fast_tm<=7.88333 then decgrp=4;
       if 7.88333<fast_tm<=12.4333 then decgrp=5;
       if 12.4333<fast_tm<=13.25 then decgrp=6;
       if 13.25<fast_tm<=14 then decgrp=7;
       if 14<fast_tm<=14.75 then decgrp=8;
       if 14.75<fast_tm<=15.7417 then decgrp=9;
       if 15.7417<fast_tm then decgrp=10;
```