Category 2 enhanced phenotyping at baseline assessment visit in last 100-150,000 participants

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Addendum to main study protocol (2 July, 2009)

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Appendix 1: Membership of the Enhancements Working Group

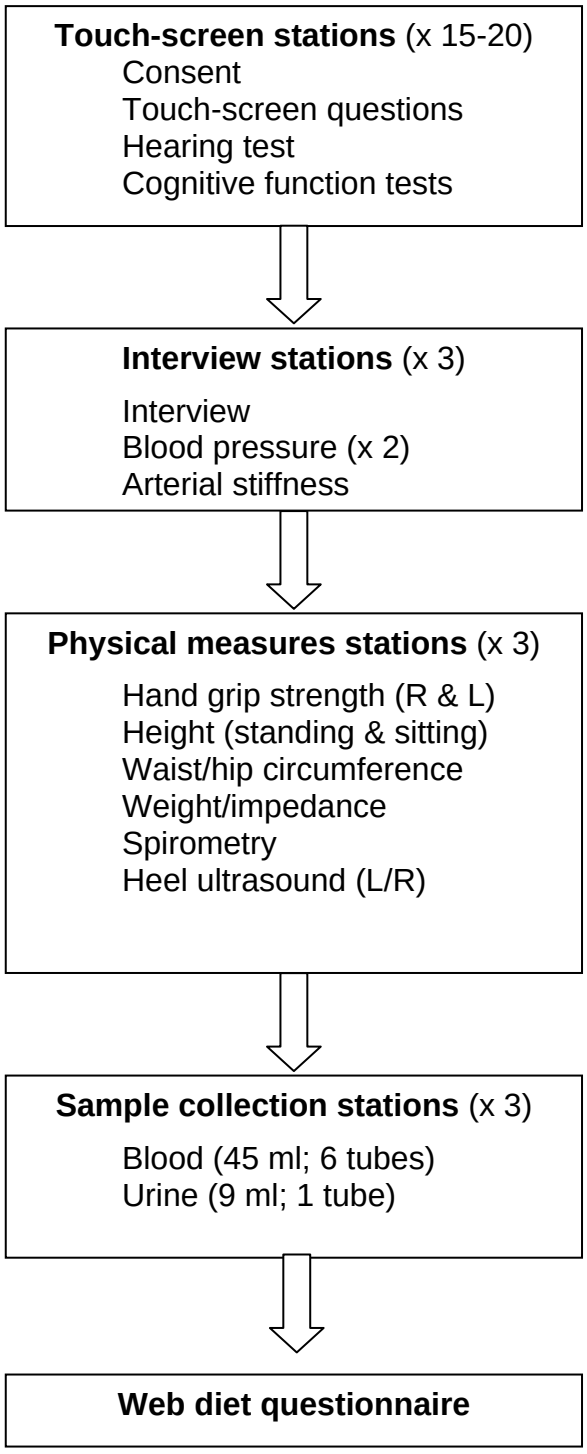
1.0 Introduction

Following piloting in 2005-2006, the UK Biobank protocol was endorsed by an International Peer Review Panel, and full-scale recruitment started in April 2007. By late 2010, UK Biobank will have recruited 500,000 men and women from the UK general population who are aged 40-69 at their baseline assessment visit. A number of questions and measures potentially associated with various health outcomes were excluded from the baseline assessment of the whole cohort for reasons of feasibility (in particular, constraints imposed by the available funding). It was, however, recognised that there were opportunities for enhancement by adding extra phenotyping in large subsets of the cohort. For, as follow-up becomes more prolonged and more cases of different conditions occur, more detailed assessments in a substantial proportion of the whole study population would become increasingly informative. This would be the case especially if such measures were more precise and more strongly related to health outcomes than those that are being made in the whole cohort. Consequently, the International Peer Review Panel endorsed, with the support of the funders, UK Biobank's proposal that a wide range of possible enhancements be investigated and this work has been taken forward by a Working Group on Enhancements (chair: Professor Paul Elliott, Imperial College, London: Appendix 1), which has consulted with a wide range of experts both in this country and beyond.

Now that UK Biobank is running smoothly, with 325,000 people recruited by July 2009, it is timely to consider introducing such enhancements. The initial round of limited enhancements (including a hearing test, additional cognitive function testing and measurement of arterial stiffness and approved by the North West REC in April 2007) are currently being implemented and this present proposal is to include a further series of more extensive enhancements to the baseline assessment visit. These additional enhancements are expected to increase the visit duration from around 1.5 to 2 hours.

1.1 Current baseline assessment visit

The main protocol describes in detail the current assessment visit, but in summary it involves participants moving through a fixed sequence of dedicated stations (including initial limited enhancements):



2.0 Additional enhancements to baseline assessment visit

The following additional enhancements are proposed for inclusion from August 2009 to the end of recruitment in 2010, allowing them to be undertaken on the last 100-150,000 participants recruited:

1. Assessment of eye related measures at a dedicated station (expected time to complete: about 10 minutes; see Section 2.1 below);

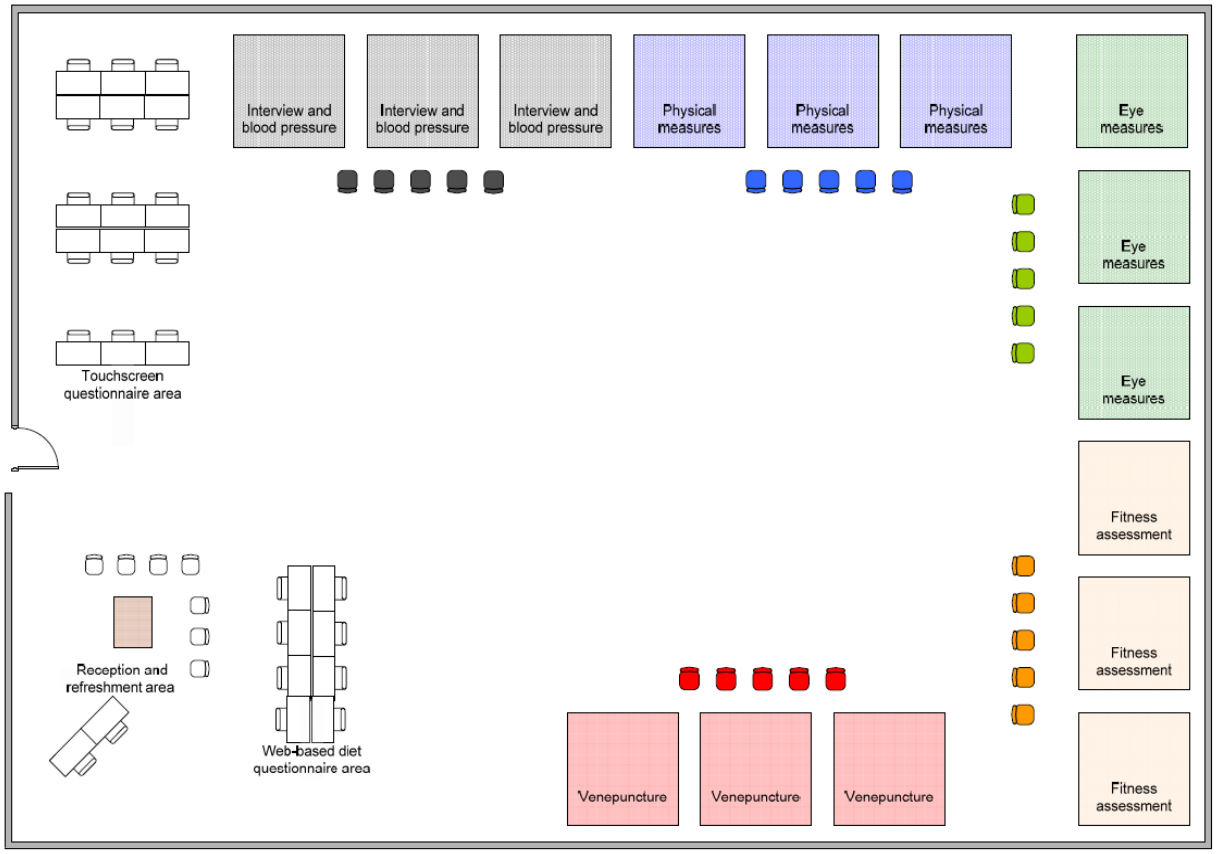
2. Assessment of physical fitness using a static bicycle with heart rate monitiring at a dedicated station (expected time to complete: about 10; see Section 2.2 below);

3. Collection of an additional 2-3ml of blood for RNA analysis at the sample collection station (No additional time required; see Section 2.3 below);

4. Collection of a 2-4ml saliva sample at the sample collection station (expected time to complete: 1-2 minutes; see Section 2.4 below);

5. Fitting of a small activity monitor (about the size of a watch) on the wrist at the end of the visit which is worn for 7 days before returning it by Freepost (expected time to complete: 5 minutes; see Section 2.5).

Below is the generic layout of a modified assessment centre incorporating these additional enhancements.



Generic layout of baseline assessment centre incorporating additional enhancements

2.1 Assessment of eye related measures

Loss of vision is a major health issue that affects about 2million people in the UK and, although the vast majority are older, it is estimated that about 80,000 working-age people are affected (Keil, 2008). Evidence suggests that over 50% of these cases are due to preventable or treatable causes (Tate et al.,2005). A significant, and increasing, number of people also live with irremediable or certifiable sight loss which is caused mainly by age-related maculopathy and macular degeneration (ARM/AMD), glaucoma and diabetic retinopathy (Bunce and Wormald, 2006). Vision loss is associated with increased risk of physical injury, emotional distress and loss of independence (Vu et al., 2005; Coleman et al., 2004; Rovner and Casten, 2002; Evans et al., 2007). Uncorrectable unilateral vision loss increases the odds of falling by threefold, and of becoming dependent for meals by sevenfold (Vu et al., 2005). The commonest registered cause of blindness and visual impairment in the UK (accounting for over 56% of the total) is age-related macular degeneration. Glaucoma (11%) and diabetic retinopathy (6%) are the next most commonly registered causes of significant visual loss. Refractive error is a fundamental determinant of visual function, and an important risk factor for

many ocular diseases (including cataract, retinal detachment and degenerative retinopathy). The age-specific incidence of blindness from these causes has increased since 1990-1991 (Bunce and Wormald, 2006). Furthermore, the eye offers a proven, uniquely accessible, means of performing qualitative and quantitative examinations of neural and vascular tissues. It is now recognized that retinal vascular characteristics are risk indicators for major systemic causes of morbidity (including hypertension, stroke, diabetes, ischaemic heart disease and all-cause mortality), even after adjustment for known risk factors.

Currently, vision is only assessed in UK Biobank by three questions on the touchscreen questionnaire about wearing glasses/contact lenses, age when first worn, and known eye diseases. Hence, it is proposed that quantitative data are obtained on visual acuity (VA), refractive error, intraocular pressure (IOP) and corneal biomechanics. In addition, the inclusion of digital photography and optical coherence tomography (OCT) would allow the qualitative and quantitative assessment of the retinal vascular system, neuro-retina and optic nerve (based on retrospective analysis of stored images from the enhanced assessment visit). In contrast to most areas of biomedical research, ophthalmology often uses data collected directly from a pair of organs (i.e. both eyes). Furthermore, unlike other paired anatomical structures (e.g. kidneys, lungs), asymmetry of function of the eyes is often immediately noticeable, worrying to the individual, and causes important and measurable effects on quality of life. A multitude of factors, both environmental and genetic, act at the level of the individual and thus have an impact on the probability of a finding occurring in both eyes (Newcombe and Duff, 1987). This has been tackled in some studies by selecting one eye per individual at random for assessment. Collecting data from one eye is statistically valid, but it is likely to be less efficient, with reductions in statistical power. Methods of analysis (such as random effects models and generalized estimating equations) can be used to deal with the problem of correlation between eyes (Murdoch et al., 1998).

Exploration of gene-gene and gene-environment interactions in eye diseases should help to clarify aetiological mechanisms that can be translated into improvements in risk profiling, as well as preventive and curative interventions. Linkage with NHS hospital activity data should permit the study of common ophthalmic surgical procedures (such as cataract). The breadth of the proposed ophthalmic measures, combined with the depth of data on lifestyle, environment, other health-related outcomes and biological samples being collected in UK Biobank, would also allow many novel associations to be explored between eye-related characteristics and systemic diseases.

2.1.1 *Visual acuity:*

Measurement of visual acuity is essential for putting other aspects of ocular phenotyping into context by determining the presence of “visually significant” disease. Visual acuity at 3 metres with best available refractive correction would be assessed with a computer-based system using LogMAR optotypes,

and a computerised, semi-automated test system programmed specifically to enhance speed and accuracy of response (Figure 2.2.2). LogMAR vision charts were developed for the Early Treatment of Diabetic Retinopathy Study (ETDRS) longitudinal study and are considered to be the reference standard for assessing visual function. This computer-based platform has already been used previously in community-based test settings. LogMAR measures are superior in terms of reproducibility and statistical analysis to the Snellen acuity measures used in UK hospital and optometric practice.

Visual acuity test using LogMAR optotypes with a computerised, semi-automated system

The prevalence of visual impairment in the UK is not well documented for the age group being studied in UK Biobank. Measures of visual acuity remain the reference standard method for assessing visual function in occupational settings and the registration of blindness or low vision. Good visual acuity is a statutory requirement for holding a driving license and, as such, even moderately impaired vision has significant financial and social implications. Mildly impaired visual acuity has also been found to be a risk factor for all-cause mortality (McCarty et al., 2001), even after adjusting for age, gender, diabetes, hypertension, smoking, heart disease, stroke and income levels (Foong et al., 2008). But, it has been suggested that this association is due to confounding by co-morbidities, risk factors, and other factors related to death, rather than being an independent biological association with vision problems or specific eye diseases (Thiagarajan et al., 2005). The inclusion of visual acuity measures would help to resolve this issue, and offer insights into their

predictive value for a wide range of conditions.

2.1.2 Refractive error

Refraction would be assessed in both eyes using a Tomey Autorefractor (see below), with information recorded about any previous refractive or cataract surgery.

Tomey RC5000 Autorefractor

Among European populations aged over 40 years, approximately one third have a significant refractive error, with myopia of greater than one dioptre accounting for the majority (Kempen et al., 2004). US estimates for the 40-65 year age group suggest that about one-half of direct eye and vision-related healthcare costs is devoted to correction of refractive error (US $3.6 billion in 2004; compared with US $2.1 billion for cataract). Refractive error is a potent risk factor for cataract, retinal detachment (both before and after cataract surgery), glaucoma and degenerative retinopathy (Harding et al., 1989; The Eye Disease Case-Control Study Group, 1993; Erie et al.,2006; Mitchell et al., 1999; Shih et al., 2006). Low myopia confers a 4-fold increased risk of retinal detachment, and high myopia a 10-fold increased risk. Myopia is associated with greater exposure to near-work stimuli (Saw et al., 2002), and 13 myopia-related genetic loci have been identified, with gene-environment interactions appearing to be highly likely, but the aetiology of refractive error in the general population remains obscure

2.1.3 Intraocular pressure and corneal biomechanical properties

IOP and corneal hysteresis would be simultaneously measured in both eyes using a Reichert Ocular Response Analyzer (see below). This device is a non-contact tonometer, which makes its use by technical staff safer and more acceptable.

Reichert Ocular Response Analyzer

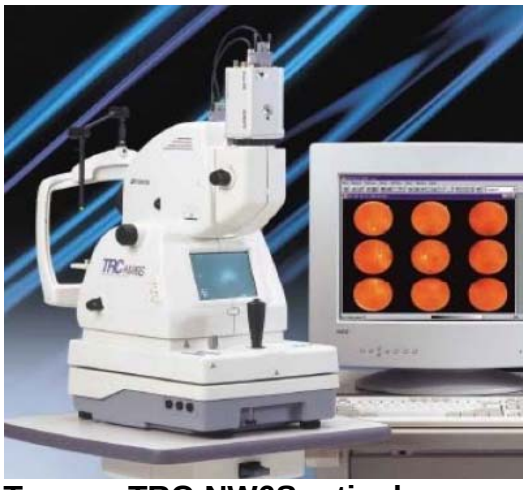
IOP is the cardinal modifiable risk factor for glaucoma, with higher IOP often reported in older people (although this is not a consistent finding). It is not clear whether these age-related difference are a cohort effect or a genuine increase with age (Nomura et al., 1999). Corneal biomechanical properties are a source of measurement error in the assessment of IOP (Ehlers et al., 1975). It has been suggested that central corneal thickness is an appropriate measure of these properties, and may be an important risk factor for glaucoma in its own right (Brandt et al., 2004). But, central corneal thickness is an unreliable guide to material properties of the cornea, which probably have a greater effect on IOP measurement (Liu and Roberts, 2005). Corneal hysteresis has been proposed as an alternative measure of tissue biomechanical properties (Luce, 2005), and is associated with progressive glaucoma (Congdon et al., 2006). Determinants of IOP are not fully understood, with only about 5% of variation directly attributed to variation in systemic blood pressure (Leske and Podger, 1983; Hennis et al., 2003; Foster et al., 2003). Short and long-term aerobic exercise is associated with a reduction in elevated IOP (Passo et al., 1987; Passo et al., 1991), possibly as a result of changes in plasma osmotic pressure (Ashkenazi et al., 1992; Martin et al., 1999). So, for example, comparisons with the detailed information that it is planned would be obtained in UK Biobank on physical activity (see Section 2.2.6) may indicate whether lifestyle modification has the potential to be an effective, and safe, non-pharmacological means of reducing glaucoma risk.

Glaucoma is a chronic, degenerative condition of the optic nerve, which ranks second only to cataract as a cause of blindness worldwide (Resnikoff et al., 2004). It is proposed that structural characteristics of the optic nerve be recorded using digital colour photography and optical coherence tomography (see below). Primary open-angle glaucoma (POAG) is the most common form of glaucoma in Caucasian people. Its prevalence increases with age, with rates that depend on the precise definitional criteria used (Wolfs et al., 2000), ranging from 0.2-0.5% in those aged 40-49 years to 3-10% in those aged 80-

89 (Wensor et al., 1998; Mitchell et al., 1996; Tielsch et al., 1991). A conservative estimate of the prevalence among people aged over 40 in the UK population would be 2%, which translates into about half a million people being affected by POAG. As a chronic condition that can cause a profound impact on the economic and social activity of those who are severely affected, glaucoma has considerable direct and 25 indirect costs (about £7,000-17,000/person/year). With rising life-expectancy, the resource implications (let alone the human costs) of caring for large numbers of elderly people with glaucoma will be significant (Khaw, 1999). The strongest, and most consistent, risk factor for POAG is increasing age (Shiose et al., 1991; Foster et al., 2000; Dandona et al., 2000; Ramakrishnan et al., 2003; Mitchell et al., 1996; Wensor et al., 1998; Mukesh et al., 2002). As discussed above, raised IOP remains the most important modifiable risk factor that is known. A positive family history has long been recognised as important (Shin et al., 1977), and studies of monogenic forms of familial glaucoma have so far identified thirteen loci associated with POAG (Stone et al., 1997; Stoilova et al., 1996; Wirtz et al., 1997; Trifan et al., 1998; Sarfarazi et al., 1998; Wirtz et al., 1999; Monemi et al., 2005). Different mutations appear to be specific to different ethnic groups, suggesting these are relatively recent events (Gong et al., 2004), but the most common mutations (in the myocilin gene) have been found in only 2-4% of cases (Fingert et al., 1999). Consequently, a polygenic mode of inheritance, with gene-environment interactions, remains a likely model in many cases (Duggal et al., 2005). It has been suggested that glaucoma may be also associated with other neuro-degenerative conditions (e.g. Alzheimer's and Parkinson's disease) offering the possibility of novel insights into their aetiology (Pache 2006).

2.1.4 Age-Related Maculopathy and Macular Degeneration

The use of the Topcon TRC-NW6S retinal camera (see below) to capture high-resolution OCT images that show "microscopic" features of the optic nerve and macula would allow more reliable determination of the causes of vision loss. Additionally, OCT would allow quantitative analysis of the spectrum of retinal and optic nerve anatomical features in the population.



Topcon TRC-NW6S retinal camera

ARM/AMD would be assessed from digital colour photographs of the retina, and from macular OCT in people with visual acuity <6/12. ARM-lipoprotein deposits called “drusen” and retinal pigmentary abnormalities in the outer retina are precursors to established AMD (neovascular maculopathy or geographic atrophy) with loss of central vision. A marked age-related increase in the prevalence of typical ARM and AMD lesions is characteristic. The disease is more common among Europeans than African-derived populations, with the Chinese having a peculiarly high rate of exudative AMD (Klein et al., 2006). Among people of European decent, AMD was present in about 2% of those aged over 50 years, rising from 0% among people younger than 55 to about 18% among those aged over 85 (Mitchell et al., 1995). Complement Factor H (CFH), which is the major inhibitor of the alternative complement pathway, accumulates within drusen and is synthesized by the retinal pigment epithelium. Common functional polymorphisms in CFH and C3 genes increase the risk of AMD in an allelic dose-response fashion (Klein et al., 2005; Yates et al., 2007), and the CFH polymorphism is widely regarded as the single most important genetic risk factor for blindness. Furthermore, abnormalities in CFH are associated with haemolytic uraemic syndrome and membranoproliferative glomerulonephritis (de Cordoba 2008). It is believed that genetic variation in regulation of complement, combined with a triggering event (such as infection), underlies a significant proportion of AMD (Hageman et al., 2005). Smoking is a significant risk factor for AMD (Tomany et al., 2004), and acts in a multiplicative manner with CFH risk polymorphisms to increase risk. The CFH variant Y402H (rs1061170:T>C, 1q32) increases the risk of both atrophic (“dry-type”) and neovascular (“wet-type”) AMD in smokers and non-smokers (Sepp et al., 2006). Currently, known genetic risk factors and smoking together explain about 60% of the population-attributable risk (PAR) of AMD (Schmidt et al., 2006). UK Biobank offers the opportunity to examine gene-gene and gene-environment interactions in those with and without these known risk factors.

In generating quantitative data on, for example, retinal nerve fibre layer thickness, UK Biobank might also be able to identify novel associations between ocular neurodegeneration (i.e. glaucoma) and central neuro-degenerative states (i.e. dementia). If present, such associations could offer new approaches to the detection and monitoring of neuro-degenerative conditions (such as Alzheimer’s disease), and provide potentially new insights into their aetiology.

Digital colour photographs of the retina could also be used to assess retinopathy and vascular geometry. Haemorrhagic and exudative retinopathy are a hallmark of diabetes, but they are also seen in 2-15% of the non-diabetic general population (Klein et al., 1994). Retinopathy (micro-aneurysms, haemorrhages and exudates) is associated with lower cognitive test scores (Wong et al., 2002b). Computer-assisted grading systems offer the potential of rapid, quantitative assessment of retinopathy and retinal vascular morphometry in the future (Gao et al., 2000; Cheung et al., 2008; Li et al., 2005), and it is already possible to carry out detailed quantitative analyses of retinal structures. Arteriolar narrowing and arterio-venous (A-V) nipping are

markers of past blood pressure levels (Wong et al., 2001). Retinal A-V calibre ratio is a risk indicator for stroke, heart disease and micro-angiopathy associated with diabetes, which persists after adjustment for cigarette smoking, plasma lipid levels and other risk factors (Wong et al., 2002a). The incidence of diabetes is higher in persons with lower A-V ratios, even after controlling for other factors. This association persists with different diagnostic criteria, and is seen even in people at lower risk of diabetes, including those without a family history of diabetes, without impaired fasting glucose, and with lower measures of adiposity (Wong et al., 2002c).

The sheer size of UK Biobank presents logistical issues in terms of the examination of large numbers of individuals, the need to use examination time as efficiently as possible, and the assessment of a series of different characteristics. It is, therefore, important to use the limited time available to maximum effect. The proposed measurements can all be performed within about 10 minutes without needing to dilate the pupil. Although they require separate devices, these could be combined in a single measurement station (which would need to be darkened for retinal imaging). Three such stations would be required in each assessment centre in order to maintain the required daily throughput of participants, each staffed by a healthcare technician trained in the assessments (plus an extra set of equipment in the “super-booth” that is used to avoid bottlenecks during busy periods).

2.2 Assessment of cardio-respiratory fitness

Cardio-respiratory fitness and physical activity are related, but independent, determinants of general health (Paffenbarger, Jr. et al., 1978; Saltin et al., 1979). Cardio-respiratory fitness also appears to be determined by inherited factors. Few epidemiological studies have included measures of cardio-respiratory fitness, but those that have indicate that it is strongly related to metabolic disease, cardiovascular outcomes and all-cause mortality (Blair et al., 1989; Albanes et al., 1989; Cole et al., 1999; Lee et al., 1999; Sui et al., 2007). Such associations are independent of key confounding factors, including obesity. However, most such studies have involved selected populations and it is unclear whether the fitness information collected was a measure of cardio-respiratory fitness itself or simply provided a better measure of physical activity than a questionnaire because of its relative stability. Small studies with objective assessment of both activity and fitness indicate that there may be separate aetiological effects of these measures on metabolic factors (Franks et al., 2004; Ekelund et al., 2007; Schmidt et al., 2008). However, those studies were not of a sufficient size to be able to investigate the impact on disease incidence.

The gold standard for assessing aerobic capacity is maximal oxygen uptake, measured during a graded exercise test to exhaustion, ideally using an individualized protocol that achieve maximal exertion after 8 to 12 minutes from the warmed-up state (Buchfuhrer et al., 1983). However, such testing is neither safe nor feasible in large epidemiological studies involving individuals with a wide range of abilities, including some with a contra-indication to

maximal exercise. Although attempts have been made to estimate fitness without individually measured exercise-response variables, this approach produce estimates with substantial uncertainty and is generally not recommended (Whaley et al., 1995). Several submaximal testing protocols and prediction equations have, however, been developed that do rank individuals fairly accurately (Aastrand and Ryhming, 1954; Paliczka et al., 1987; Noonan and Dean, 2000; Uth et al., 2004; Brage et al., 2005; Brage et al., 2007). For example, the 8 minute ramped step-test has been widely used in fieldbased studies (Brage et al., 2005), including the current household-based Health Survey for England in which 337 of the first 400 eligible participants completed at least 6 minutes of stepping after pre-screening. This test can be conducted relatively quickly (~15 minutes; including instructions, setup, step-test and recovery) in a confined space using highly portable, but technologically-advanced, equipment to obtain an ECG trace (one lead) and a synchronised movement waveform signal for uni-axial acceleration. The philosophy behind this method is to maximise the information content per unit time to provide maximal flexibility for the subsequent modelling stage of the inference scheme (Brage et al., 2007).

A step-test may not, however, be the most suitable exercise modality for the assessment of fitness in certain settings because work load is not always well standardised during stepping (Noonan and Dean, 2000). Step-testing is also less safe for people who experience balance problems (e.g. due to exercise-induced dizziness), which is also an issue for treadmill tests but much less so for bicycle tests. Aastrand described a 6-min steady-state sub-maximal cycle ergometry test (Aastrand and Ryhming, 1954) which has undergone substantive validation with some modifications (Siconolfi et al., 1985; Legge and Banister, 1986). In general, graded-exercise protocols yield more information about work capacity, and ramped protocols may yield even more than steady-state protocols for the same test duration (Fitchett, 1985). Exertion during ramped protocols is generally perceived by participants to be less than that of steady-state protocols, despite covering a wider exercise intensity range (Will and Walter, 1999), thus favourably shifting the balance between test discomfort for the participant and phenotypic information obtained. In one study, monitoring heart rate during a ramped cycle ergometry protocol with starting load of 25W, ramp slope of +10W/min, and maximum duration of 16 min with 1.5-min recovery explained 66% of the between-individual variation in fitness (Brage, unpublished observations). It is proposed to utilise this ramped cycle ergometry protocol in UK Biobank.

The proposed assessment of cardio-respiratory fitness would take about 10 minutes (5-8 minutes for the test plus 2 minutes each for preparation and recovery). A pre-test screen would be used to exclude people with contraindications to the test (e.g. angina, aortic stenosis and dysrrhythmias). Based on previous experience with the Canadian Home Fitness Test, such pre-screening has been used successfully without the occurrence of any adverse events in population-based studies of unselected volunteers of comparable age to those in UK Biobank. The ergometer will be programmable with respect to work load (including ramp protocols) and robust with respect to

cadence (magnetically-braked ergometer or equivalent technology: see below). Time-stamped synchronised data will be stored on: (i) prescribed work load; (ii) measured cadence (>0.1 Hz resolution); and (iii) limb ECG leads. The starting work load and ramp slope on the ergometer (see below) will be quasi-adapted to the individual, based on age, gender, weight and resting rate heart up to 65% of the calculated maximal predicted power.

Seated ergometer for testing physical fitness

This assessment of cardio-respiratory fitness would require the addition of three dedicated stations in each assessment centre, with a healthcare technician, seated exercise machine and automated ECG device in each station (plus an extra set of equipment in the “super-booth” that is used to avoid bottlenecks during busy periods).

2.3 Collection of whole blood for the isolation of RNA

The blood and urine samples currently being collected in UK Biobank will provide a good representation of the genome, proteome and metabolome of the participants (Elliott and Peakman, 2008). However, none of these samples will allow the study of transcription profiles in the various cell types of the blood, and the potential to study the epigenome is limited to methylation and acetylation profiles of DNA and associated histone complexes.

The transcriptome is important because control exerted at the RNA level can have profound phenotypic effects. Rate of transcription, differential stability, RNA transport, splicing and splice variation, and secondary structure can all dramatically affect the concentrations and isoforms of many proteins. Splice variation alone can produce proteins from the same gene with as little as 50% homology. On average, each human gene produces three different proteins due to splice variations; but, for example, about 1000 variant neurexin proteins can be derived from the three neurexin genes. It is an important question whether transcription profiles in blood are representative of transcription in other tissues, particularly in pathological conditions. Data are relatively sparse, but a study comparing post-mortem transcription profiles in

the brains of patients with Huntington's disease (Borovecki et al., 2005) versus those in their blood demonstrated that alterations of gene expression in both tissues correlated well. In combination with genomic information, transcription profiles from high-throughput chip-based techniques may well be a powerful tool in understanding disease aetiology. For example, leucocyte transcription profiles have been used to assess the systemic consequences of risk factors for vascular disease (Ardigo et al.,2007) and to diagnose idiopathic arthritis in juveniles (Allantaz et al., 2007).

Epigenetic processes are fundamental in normal and disease processes within the cell through their involvement in DNA packaging and transcription regulation. They include histone modification, histone positioning, methylation of the DNA and the interaction with the genome of small and non-coding RNA molecules. These mechanisms interact with transcription factors and other DNA-binding proteins to regulate gene expression involved in embryonic development, differentiation and cell identity. They are also involved in responses to environmental signals, such as hormones, nutrients stress, and cell and DNA damage. Epigenetic processes are heritable in somatic cells but can be modified by drug treatments, which has significant implications for the diagnosis and treatment of disease. In addition, recent technological advances (such as microarrays and ultra-high-throughput sequencing) have enabled much greater detailing of the epigenome (particularly chromatin modifications, cytosine methylation and non-coding RNAs), which offers the potential to examine the epigenomes of large numbers of subjects (American Association for Cancer Research Human Epigenome Task Force and European Union Network of Excellence, Nature 2008).

Inclusion in UK Biobank of a sample suitable for RNA extraction would allow the study of transcription profiles in the various cell types of the blood and of a wider range of epigenetic processes. It was recommended by UK Biobank's sample handling subgroup during the planning phase, but was excluded on the basis of the high cost of collecting such samples at that time (which it is anticipated could now be reduced substantially due to the lower cost of the collection tubes).

During the current assessment visit, it would be straightforward to incorporate the collection of an extra 2-3 ml of blood in a form suitable for isolating RNA by adding a vacutainer containing 4ml of a quaternary ammonium salt. This lyses the cells and forms a precipitate with nucleic acids which is stable at room temperature for up to four days. The manufacturers (PaxGene) generally recommend that the RNA is purified (using an affinity column) within that period, but that would not be practicable or affordable in UK Biobank. Instead, it is proposed that this blood-precipitate solution would be aliquoted into 6 x 1ml aliquots and stored in liquid nitrogen. Subsequently, these samples could be retrieved for selected participants (i.e. as for the planned nested case-control comparisons with other assays) and used for RT-PCR studies directly (with DNAse steps included) or following RNA purification. Preliminary data from the manufacturers indicate that RNA extracted from

cryopreserved whole blood after 2 years at -80oC is equivalent to RNA extracted before cryopreservation.

2.4 Collection of saliva

The addition of saliva collection in UK Biobank is likely to provide a wide range of useful information that will not be provided by the blood and urine samples that are currently being collected. Saliva is formed from contributions of the salivary glands and serum exudates principally from the gingival margins or damaged mucosa. As well as being an indicator of oral health, it is a reservoir of analytes from systemic sources that reach the oral cavity through various pathways (Oppenheim et al., 2007). The molecular composition of saliva reflects tissue levels of therapeutic, hormonal, immunological and toxicological molecules. It contains several important cellular (such as epithelial cells, neutrophils, bacteria) and noncellular components (such as glycoproteins, peptides, RNA, DNA, electrolytes, lipids, steroid and protein hormones, and other small molecules). Colonizing bacteriaand other microorganisms can be identified (e.g. streptococci, lactobacilli, staphylococci and corynebacteria, with a great number of anaerobes, especially bacteroides), levels of innate and adaptive mucosal defence factors can be quantified, and products of chronic disease at mucosal surfaces can be assayed. Saliva has a major advantage over blood and urine in that it can provide data on events at mucosal surfaces, which may provide a surrogate for other such fluids (e.g. vaginal, gastric, nasal).

Collection of a saliva sample may be valuable for the measurement of a number of specific dietary biomarkers. For example, dietary nitrate is absorbed from the stomach and the proximal small intestine into the systemic circulation, and is concentrated in the saliva by a factor of about 10 (Webb et al., 2008). It is converted by bacterial anaerobes in saliva to nitrite, which has been suggested may be responsible for the blood pressure lowering effect of vegetables rich in nitrates. Further developments in metabolomic analysis of saliva using NMR may be valuable for the study of dietary biomarkers (Walsh et al., 2006). The number of salivary biomarkers is increasing rapidly and many of these markers appear to be unique to saliva. For example, a list of 1,939 salivary proteins has recently been published (Denny et al., 2008) and around two-thirds of these proteins in the salivary proteome have not been found in blood. This may lead to the discovery of protein "signatures" indicative of oral and systemic diseases (such as periodontal disease, viral and bacterial infections, oral cancer, renal disease, asthma and cardiovascular disease: Malamud and Nielbala, 2007). Similarly, around 200 mRNAs has been identified in the salivary transcriptome (Park et al., 2006) and a recently compiled catalogue has identified around 700 oral bacterial species (Human Oral Microbiome Database).

A saliva sample may be particularly useful for studying biomarkers associated with diseases of the mouth, such as periodontal disease leading to tooth loss and oral cancers. For example, a case-control analysis comparing women with significant alveolar bone loss over a 5 year period with age-matched

controls with no bone loss found significant associations with specific salivary inflammatory biomarkers, including hepatocyte growth factor, interleukin-1ß and osteonectin (Scannapieco et al., 2007). Turning to oral cancer, salivary proteomic analysis has recently identified a number of proteins (e.g. catalase and MRP14, which have been implicated in other cancer types) that are significantly elevated in patients with oral squamous cell carcinoma compared to matched controls, and a multicentre study is currently validating a "signature" of 7 mRNAs in saliva for the detection of oral cancer (Hu et al., 2008; Li et al., 2004). Furthermore, a number of studies have found periodontal disease and tooth loss to be independent risk factors for cancer of the head and neck, oesophagus, kidney and pancreas (Hiraki et al., 2008; Michaud et al., 2008), which has led to the suggestion that periodontal disease may be a marker of a susceptible immune system. Several mediators of the inflammatory response (including cytokines, prostaglandins and various growth factors) can be readily detected in saliva, and it has been postulated that they may be involved in the link between inflammation and cancer (Hussain and Harris, 2007).

Turning to other systemic diseases, poor dental health has been associated with acute myocardial infarction after adjustment for known risk factors, including age, social class, smoking, blood lipid concentrations and diabetes (Mattila et al., 1989). Subsequent studies have found associations of periodontal disease and tooth loss with carotid artery intima-media thickness and plaque which persist after adjustment for conventional risk factors (Beck et al., 2001; Desvarieux et al., 2003). Analysis of periodontal bacterial species in subgingival plaque samples have found that overall bacterial burden, as well a number of specific bacterial species responsible for periodontal disease, are associated with carotid intima-media thickness (Desvarieux et al., 2005).

A 2-4 ml sample of saliva would be collected into a bar-coded tube without preservative at the sample collection station and then chilled to 4°C. This sample would be aliquoted into 2 x 1ml aliquots at the coordinating centre laboratory and stored in liquid nitrogen.

2.5 Activity monitoring using an accelerometer device

Physical activity and cardio-respiratory fitness are related, but independent, determinants of health that are typically poorly measured in most epidemiological studies (Paffenbarger et al., 1978; Saltin et al.,1979). Physical activity is a complex behaviour with several underlying dimensions (including energy expenditure, aerobic intensity, load-bearing and strength enhancing activity) that may be related to different health outcomes. Physical activity questionnaires collate information about self-reported behaviour, and the addition of further questions about physical activity to the touch-screen system in UK Biobank during early 2009 should provide improved information in the key domains of transport, domestic life, work and recreation. One of the key dimensions of activity is the pattern and total volume of energy expenditure. Although attempts have been made over many years to quantify energy

expenditure from questionnaires covering the reported duration, frequency and intensity of activity by reference to compendia of the energy costs of specific activities, this approach is very imprecise. The demonstration of associations between questionnaire-derived measures of activity and major chronic disease outcomes in the face of this imprecision suggests that the true underlying relationships are much stronger. Advances in technology have made it possible to assess physical activity, and its associated energy expenditure, more accurately using objective non-invasive methods.

The inclusion of such a measurement in UK Biobank would allow quantification of the relationship of both type and amount of physical activity with major chronic disease outcomes (Kriska, 2000; Besson et al., 2008) and with less common diseases for which the current evidence of association with activity is uncertain (e.g. site-specific cancers: Albanes et al., 1989). The use of a more precise measure of activity should greatly enhance the ability to investigate interactions between genes and physical activity on these chronic diseases, and also to assess direct associations between genetic variants and physical activity itself (Wong et al., 2003). For example, it has proved possible to demonstrate an interaction between the effects of the FTO gene and physical activity on obesity risk using an objective measurement of activity (Rampersaud et al., 2008) in a much smaller study than with a questionnaire-based method (Andreasen et al., 2008). Finally, inclusion of objectively measured activity would provide opportunities for investigating the determinants of behaviour, ranging from genetics to social and environmental factors. This could be of considerable value to current public health strategies.

Improving the accuracy of physical activity assessment would also help to clarify the dose-response relationship between activity and various health outcomes and, specifically, which dimension of activity (type, intensity, frequency, duration, or total volume) is most strongly related to health (Wareham and Rennie, 1998). Currently available methods have strengths and limitations with regard to precision, costs, practicality, and degree of invasiveness (Rennie and Wareham, 1998; Corder et al., 2008). Physical activity questionnaires are generally low cost, relatively easily administered, and have low participant burden, but are poor at estimating physical activity energy expenditure (PAEE) on an individual level (Albanes et al., 1990; Rennie and Wareham, 1998; Wareham et al., 2002; Masse et al., 2002; Ainslie et al., 2003). The gold standard method of doubly-labelled water allows quantification of total energy expenditure over a prolonged period of time, but it is expensive and cannot quantify dimensions of physical activity, such as intensity and frequency (Speakman, 1998; Ainslie et al., 2003). Consequently, there has been considerable interest in developing novel techniques for objectively measuring physical activity in population-based studies.

Currently used motion sensors and heart rate (HR) monitors are capable of storing information about activity intensity over several days with time resolutions in the range of seconds or minutes. But, the validity of existing inference schemes using this level of input information is compromised by different factors. Motion sensors demonstrate highly variable relationships to

physiological activity intensity for different activities (Montoye et al.,1983; Sekioka et al., 1998; Jakicic et al., 1999; Nichols et al., 1999; Bassett, Jr. et al., 2000; Hendelman et al., 2000; Ekelund et al., 2001; Ekelund et al., 2002a; Strath et al., 2003; Ekelund et al., 2003; Brage et al., 2003b; King et al., 2004; Treuth et al.,2004). They are most suitable for the assessment of low to moderate levels of physical activity intensity, while HR monitoring is most suitable for the assessment of moderate to vigorous levels of physical activity intensity. The main limitation with HR monitoring is that it is affected by factors other than physical activity (e.g. stress), and the method relies more heavily on individual calibration (Andrews, 1971; Ceesay et al., 1989; Livingstone et al., 1990; Li et al., 1993; Strath et al., 2000; Ekelund et al., 2002b; Brage et al., 2005; Strath et al., 2005; Brage et al., 2007)

Methods that could integrate physiological and motion detection systems have been identified as a promising research area (Bassett, 2000; LaMonte and Ainsworth, 2001), and previous studies have demonstrated the utility of combined HR and movement sensing to improve the precision of PAEE (Avons et al., 1988; Haskell et al., 1993; Moon and Butte, 1996; Luke et al., 1997; Treuth et al., 1998; Rennie et al., 2000; Strath et al., 2001; Strath et al., 2002; Brage et al., 2003b; Brage et al., 2004; Brage et al., 2005; Strath et al., 2005; Thompson et al., 2006; Brage et al., 2007; Crouter et al., 2007). But, although this combination may be viewed as an “epidemiological gold standard” for the assessment of habitual activity intensity, its current implementation using ECG electrodes has three major drawbacks: (i) a fraction (~10%) of the population will experience a minor skin rash as a reaction to the electrodes; (ii) physiological signals (e.g. ECG) are inherently prone to measurement noise that requires pre-processing of the collected information before interpretation (Stegle et al., 2008); and (iii) current devices are relatively costly. For these reasons, it is not proposed to measure habitual physical activity by combined HR and movement sensing.

The use of accelerometry is becoming increasingly common and has proven feasibility in large-scale epidemiological studies, such as NHANES (Troiano et al., 2008). But, that particular study used the Actigraph sensor, which does not directly measure acceleration (Brage et al., 2003a). Indeed, most commercially available accelerometers do not store the measured signal in its raw resolution, but instead perform onboard processing and data reduction to meet the memory and power constraints (Corder et al., 2008). The measure that is most commonly extracted is average absolute magnitude, although an increasing number of models now also include movement frequency. As a consequence, although the information obtained is quantitative in nature, it is device-dependent and not readily interpreted using established biomechanical models (Brage et al., 2003b; Rowlands et al., 2007). Moreover, such monitors are typically worn on the hip, usually attached to a belt, which makes them less popular with women and likely to be taken off when clothes are removed. New generation accelerometers use seismic Micro-Electro-Mechanical Systems (MEMS) technology which responds linearly to acceleration (including gravity) within their dynamic range (e.g. +/- 6g). This property means that sensors may be calibrated by very simple calibration procedures

that can be done post-manufacturing by the investigator without the use of dynamic spinning (Lukowicz et al., 2004) and, potentially, may be worn on the wrist. Furthermore, advances in memory and battery technology now allow continuous sampling (e.g. 80 Hz) of all three dimensions of acceleration and storage of this waveform information over 7 days. An important attraction of this approach is the expression of the collected information in SI units of acceleration, which not only enables interpretation of the collected information in a biomechanical framework, but also makes the findings less dependent on a particular company's device.

Several validation studies have shown that waveform acceleration signals allow better estimation of the type of physical activity performed. The accuracy of this estimation depends on: (i) the positioning of the sensor; (ii) the abundance of the collected information; (iii) the signal features extracted; (iv) the type of classification algorithm used; and (v) the way in which the classification algorithm is "trained". By contrast to the restricted measures (magnitude and frequency) typically provided by traditional accelerometers in pre-set time intervals, the characteristics that can be derived from waveform accelerometry do not need to be pre-defined (e.g. they might involve standard deviation, kurtosis, entropy, cross-axis correlation, and numerical integration to speed and position) and can be obtained in flexible time windows short enough to comply with the Nyquist-Shannon sampling theorem. Although the field of physical activity classification research spans more than ten years, it has so far mainly focussed on the application of this technology in relatively small studies of short duration, with limited consideration of the feasibility of methods for monitoring habitual activity. Early validation studies have demonstrated the ability of accelerometers using waveform acceleration to infer which type of activity is being performed (Veltink et al., 1996; Bao and Intille, 2004; Parkka et al., 2006). This may overcome the main disadvantage of traditional accelerometry information, which is its variable relationship to physiological activity intensity across different activity types.

Progress on transferring waveform acceleration methodology to epidemiology has recently been made by the development of the GENEA accelerometer by Unilever (See below). This small (12x29x37mm), light (17g), and waterproof accelerometer contains seismic acceleration sensors constructed with MEMS technology. Pilot studies indicate that this monitor is well tolerated by study participants when worn on the wrist (MRC Epidemiology Unit, unpublished observations). Following several iterations of the software for initialising and downloading the information, this system is now sufficiently robust to ensure collection of accelerometric information for 7 days without the participant being able to affect the data integrity. Consequently, we propose using this wrist-worn accelerometer and physical activity pattern recognition system in UK Biobank.

GENEA wrist-worn accelerometer

At the end of their baseline assessment visit, the last 150,000 participants recruited would be asked to wear an accelerometer for the next 7 days and then to return the device in the pre-paid envelope provided. In addition, it is planned that we write to all previously recruited participants to enquire whether they would be willing to wear this wrist-worn accelerometer for 7 days (as in a study conducted successfully in Tasmania; Dwyer et al., 2007). Those who agree would be mailed an accelerometer with instructions on how to use it and mail it back after wearing it for a week. On receipt at the coordinating centre, the data would be extracted and the devices re-used.

An ongoing programme of validation studies is ongoing (conducted Prof Nick Wareham and Dr Soren Brage, Cambridge) to demonstrate the ability of this wrist-worn accelerometer to measure physical activity energy expenditure and to enhance its ability to discriminate between different forms of activity by pattern recognition. This will include a doubly-labelled water study to examine the capability of assessment methods based on wrist-worn accelerometry versus current sensors (such as Actigraph) to normalise energy intake appropriately (Jakes et al., 2004). It is expected that the wrist-worn accelerometer will, at the very least, be shown to capture movement to the same degree as current sensors that are worn on the hip (but are less likely to be worn continuously throughout a 7-day period). However, it is anticipated that the development of the interpretative software will greatly enhance the information produced from this wrist-worn accelerometer, and will open up future avenues for research that would not be possible if more limited techniques were employed. Developing a robust classification algorithm for activity type requires the investigation of many types of physical activity and optimization for multiple subgroups within different populations. UK Biobank will, therefore, build a data repository to which multiple research centres can upload accelerometer data accompanied by a specification of the type of physical activity performed and basic characteristics of participants (weight, age, gender, and height). Alongside this repository, an open-source community will be established to facilitate the development of algorithms for the classification of the type of physical activity based on data in the repository. This approach should speed the development of classification algorithms, as has been the case with the inference schemes for ECG waveforms developed within the PhysioNet resource (www.physionet.org).

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Appendix 1: Membership of the Enhancements Working Group

Professor Paul Elliott; Imperial College, London (Chair)

Professor Mark Caulfield; Queen Mary, University of London
Dr John Gallacher; University of Cardiff
Professor Alan Silman; University of Manchester
Professor Nick Wareham; University of Cambridge
Dr Ioanna Tzoulaki; Imperial College, London
Professor Rory Collins; University of Oxford & UK Biobank
Dr Tim Sprosen; UK Biobank