Inflammatory markers
Focussing on the variables found in secondary data resources in the UK

Meena Kumari

CDT module 2: January 2020

Aims of today

- Understanding of the biological data available in the secondary data resources in the UK

- Insight into the choices of biomarkers available in these resources

- Insight into the issues needed to be considered when analyzing these data

Immune system

- ## What is the immune system?
  - Multi-layered defence system who's function is to protect individuals from infectious organisms, microbes and toxins.

  - It must be able to:
    - Distinguish between "self" and "non-self"
    - Destroy foreign antigens without damaging the host
    - Record antigenic experience so immune response is more efficient/effective later

                                        (Clough and Roth, 1998)

Inflammatory markers

- Brief overview of the immune system

- Introduction to the 'messengers' in the immune system – cytokines

- Overview of research into the cytokines and social or psychological factors in man

- Other aspects of immune function

Immune system

- Background
  - Skin, saliva, acid in stomach, mast cells in nasal passages, throat, lungs

- Innate; non specific defence;
  - Phagocytes (macrophages, monocytes)

- Active; specific defence
  - Antibodies (immunoglobulins)

Immune system

– Early defence systems (natural immunity)

- begins on the outside with the skin, which acts like a fortified wall to keep bacteria out of the body. The skin produces anti-bacterial chemicals that kill most invaders on contact. As long as it remains unbroken, the skin is impervious to bacteria and viruses.
- Mucous, saliva and tears also contain anti-bacterial enzymes that protect the body from outside invaders.

Immune system

- Inside the body, the defence system is made up of multiple components that work together to fight disease.
    - The lymph nodes and spleen act as filters to keep contaminates out of the blood.
    - The thymus and bone marrow are the "factories" that produce the immune system's weapons. The thymus produces T-cells which are vital to building the immune system in its early development, while the bone marrow produces blood cells.
    -

Immune system

- Natural immunity is 'all purpose' immunity that protects against all types of cells.
  - This early generalised response is called 'inflammation'.
  - Localised response at site of injury by granulocytes – molecules like macrophages and neutrophils. These make communication molecules call cytokines
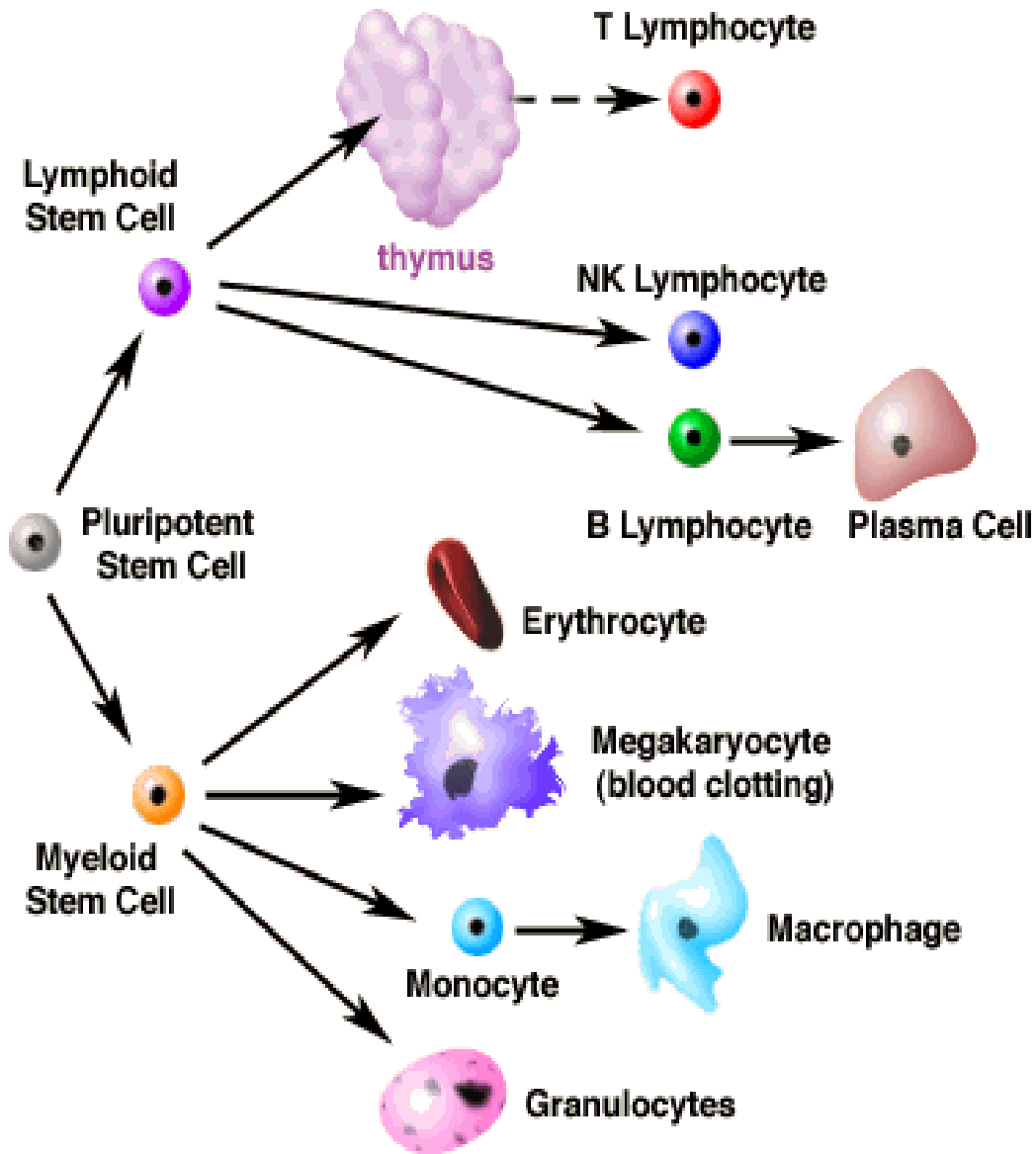- Specific immunity from lymphocytes; cells that produce antibodies.

Immune system

- If a foreign invader manages to get past all the early defence systems, the body's sensors will detect its presence and send out an alert.
  - Antibodies are sent to the site to attack and kill the germ. It is this operation which most often produces the symptoms of an illness.

Immune system

- Inoculations (vaccinations) are used to help the body identify unknown germs and develop antibodies capable of stopping future infections.

Lymphoid Stem Cell

T Lymphocyte

thymus

NK Lymphocyte

Pluripotent Stem Cell

B Lymphocyte   Plasma Cell

Erythrocyte

Megakaryocyte (blood clotting)

Myeloid Stem Cell

Macrophage

Monocyte

Granulocytes

Anything that causes an immune response is called an *antigen*. An antigen may be harmless, such as grass pollen, or harmful, such as the flu virus. Disease-causing antigens are called *pathogens*. The immune system is designed to protect the body from pathogens.

In humans, the immune system begins to develop in the embryo. The immune system starts with hematopoietic (from Greek, "blood-making") stem cells. These stem cells differentiate into the major players in the immune system (granulocytes, monocytes, and lymphocytes). These stems cells also differentiate into cells in the blood that are not involved in immune function, such as erythrocytes (red blood cells) and megakaryocytes (for blood clotting). Stem cells continue to be produced and differentiate throughout your lifetime.

Hematopoietic stem cells produce cells in blood and lymph

Adapted from *Biology of the Immune System*, JAMA 278 (22)

By the time a baby is born, the immune system is a sophisticated collection of tissues that includes the blood, lymphatic system, thymus, spleen, skin, and mucosa.

Immune system, social and psychological processes

- Cytokines: messengers within the immune system
  - Cells of the immune system secrete soluble chemical messengers. These messengers have effects that are distant from the cells that secrete them
  - Receptors for these molecules are found in the nervous and endocrine systems
    - →**"PsychoNeuroImmunology"**
   - Besedovsky et al., 1983
      - Besedovsky and Del Ray, 1989

Immune system, social and psychological processes

- Cytokines;
  - eg Interleukins such as Interleukin-1 (IL-1); Interleukin-6 (IL-6), Tumour Necrosis Factor-α (TNF-α)
    - IL-6 acts most like an endocrine factor; that is effects occur at sites distant to the cells which produce it.

  - Made by Thymus gland and macrophages

Immune system, social and psychological processes; lifecourse perspective

- Intimate bi-directional connections between the immune system and endocrine function

- EARLY LIFE EFFECTS:
    - Infection in early life leads to life long changes in hypothalamic-pituitary-adrenal axis (Shanks et al., 1995) and increased anxiety and behavioural stress response (Shanks et al.,2000).

Immune system and 'stress'
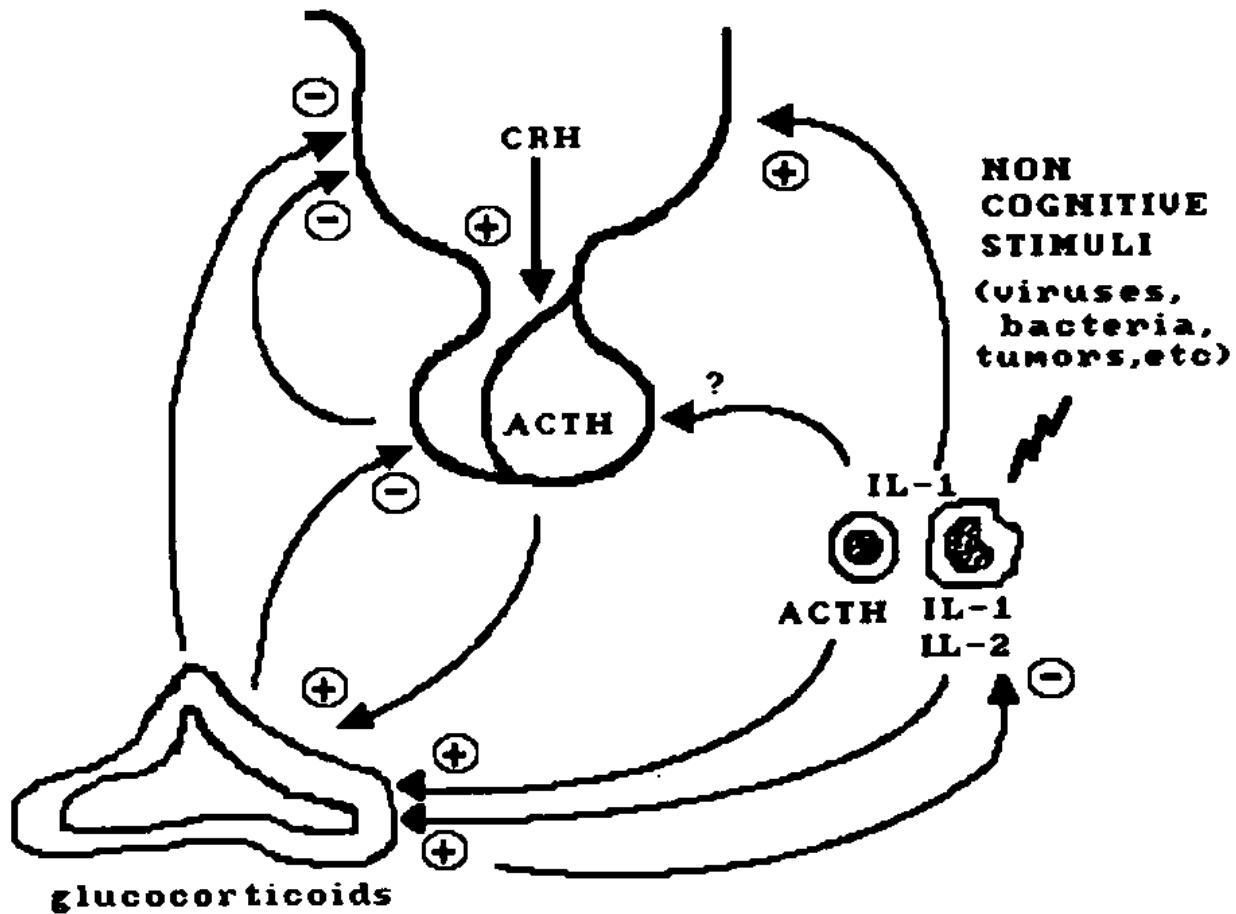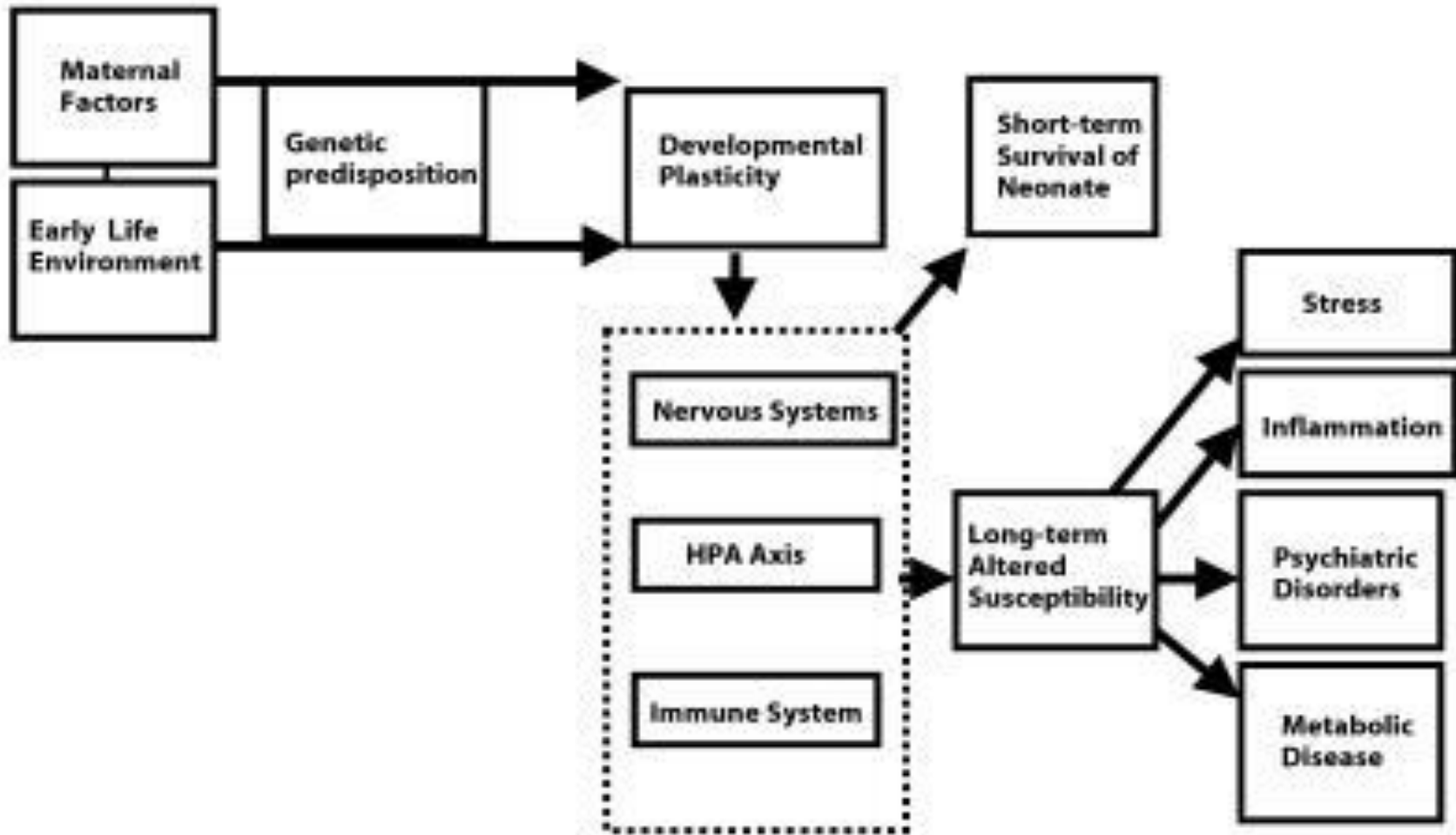
Schematic representation of the impact of maternal and early life environment factors altering neuroendocrine development. Developmental plasticity, which allows the organism to contend with environmental pressures to survive as a neonate, may alter predisposition to disease over the long-term.

Inflammatory markers

- What is happening in man?

- How do we measure immune function?
  - Inflammatory markers
    - C-reactive protein
      - Protein release by the liver activated by Il-6
    - White blood cell count
      - Mixture of cells in blood
    - Interleukin-6
    - Others – fibrinogen (also released by liver; additional coagulant activities); immunoglobulins (antibodies)

CLOSER studies

System	Biomarker	ALSPAC	UKHLS*	SWS	NCDS*	NSHD	HCS
Inflammatory/haemostasis							
	C-Reactive Protein, CRP	✓	✓		✓	✓	✓
	Fibrinogen		✓		✓		
	Immunoglobulin E, IgE	✓			✓		
	Interleukin-6, IL-6	✓				✓	
	Tissue Plasminogen Activator, TPA				✓	✓	
	Von Willebrand factor, VWF				✓	✓	
	Platelet count			✓		✓	
	Red Blood Cell, RBC, count			✓		✓	
	White Blood Cell, WBC, count			✓		✓	

Immune system, social and psychological processes; lifecourse perspective

- Associations of cytokines with social position across the lifecourse
- Associations with 'sickness behaviours' and depression
- Associations with disease; heart disease

Childhood maltreatment and inflammatory factors in adulthood
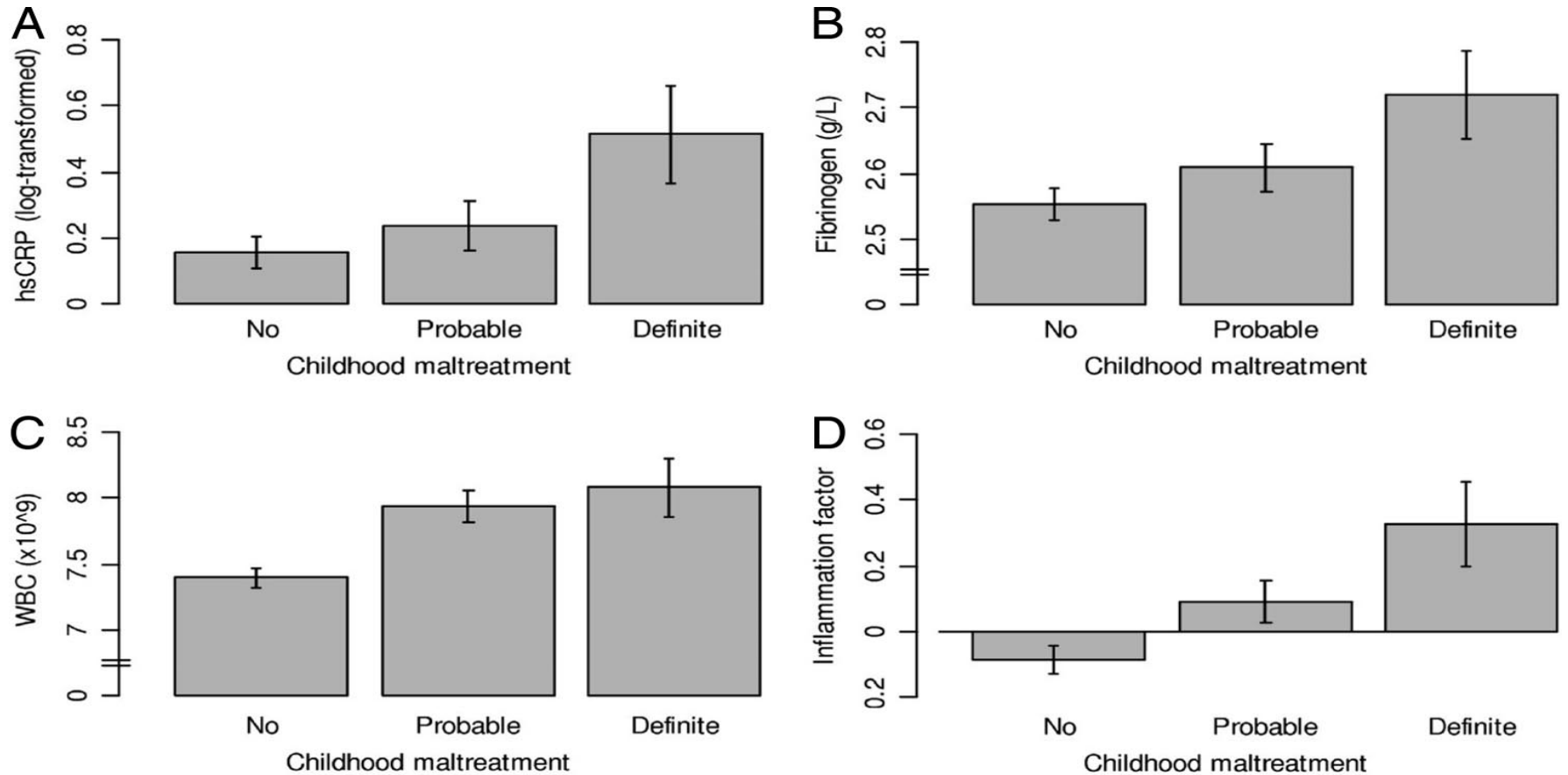
Source Danese et al PNAS 2007, 104, 1319

Inflammatory markers, social and psychological processes; lifecourse perspective

C-reactive protein (mg/liter)	Men			Women		
	Social class at birth	Social class at 23 years	Social class at 42	Social class at birth	Social class at 23 years	Social class at 42 years
Social class 1	0.89 (2.77)	0.91 (2.68)	0.99 (2.68)	0.95 (2.85)	1.06 (3.18)	1.11 (3.15)
Social class 2	0.96 (2.53)	1.00 (2.69)	1.11 (2.72)	1.09 (3.09)	1.14 (2.97)	1.15 (2.91)
Social class 3	1.12 (2.65)	1.15 (2.64)	1.14 (2.60)	1.26 (3.12)	1.52 (3.06)	1.36 (2.97)
Social class 4	1.16 (2.65)	1.23 (2.61)	1.17 (2.94)	1.26 (2.94)	1.35 (3.00)	1.33 (3.06)
p for trend	<0.001	<0.001	<0.001	<0.001	<0.001	0.005

•Social class 1, professional and managerial; social class 2, nonmanual; social class 3, manual; social class 4, unskilled.

 Geometric mean values are presented.
 Numbers in parentheses, standard deviation.

Inflammatory markers

- Difficulties with assessment in man:

  - Adipose tissue (especially central) makes cytokines (Coppack et al., 1997)
    - All assessments of cytokines and social processes complicated by associations with obesity
  - Local cytokine production can occur in response to damage, eg in atheroma

Immune system, social and psychological processes; lifecourse perspective

Depressed people have higher plasma levels of inflammatory markers

(Dentino *et al.*, 1999; Penninx *et al.*, 2003; Tiemeier *et al.*, 2003; Ford, Erlinger, 2004; Liukkonen *et al.*, 2006; Cyranowski *et al.*, 2007; Dantzer, Kelley, 2007; Ranjit *et al.*, 2007; Bremmer *et al.*, 2008;

Gimeno *et al.,* 2008)

Longitudinal analyses

- Findings from Whitehall II suggest that inflammatory markers (CRP, IL-6) are associated with the development of depressive symptoms rather than vice versa using collected over a 12year period

- These data support the notion that cytokines are causal to depressive symptoms rather than the other way around. (Gimeno et al., 2009)

Cytokines and 'sickness behaviour'

CNS effects of cytokines	Non-specific symptoms of sickness
• General malaise	• Feeling sick
• Decreased activity	• Loss of energy or fatigue
• Decreased social investigation	• Loss of interest in usual activities
• Decreased food and water intake, weight loss	• Poor appetite and significant weight loss
• Sleep changes	• Sleep changes
• Fever	• Fever

Inflammatory markers and disease

- 'innocent bystander'
  - Evidence unclear on whether cytokines are aetiological to the development of disease or are reflecting underlying atherosclerotic load

  - Evidence from Mendelian Randomisation suggests that this may be different for CRP than IL-6- further work needed

(Davey Smith et al., Ann Int. Med. 2006; 45, Danesh et al., PLoS Med. 2008)

Other aspects of immune system and social processes

- Difficult to measure 'immune function' in large scale epidemiological research
  - No easy way to administer method amenable to large scale work
  - Evidence available using more focussed experimental work

- For example:
  - susceptibility to common cold
  - Reaction to vaccinations

Volunteers 'infected' with cold Virus

Observed for infection and development of Cold symptoms
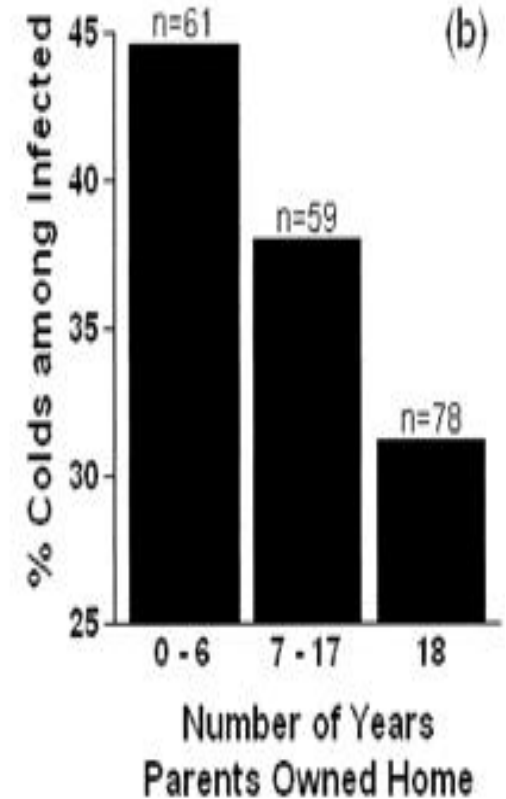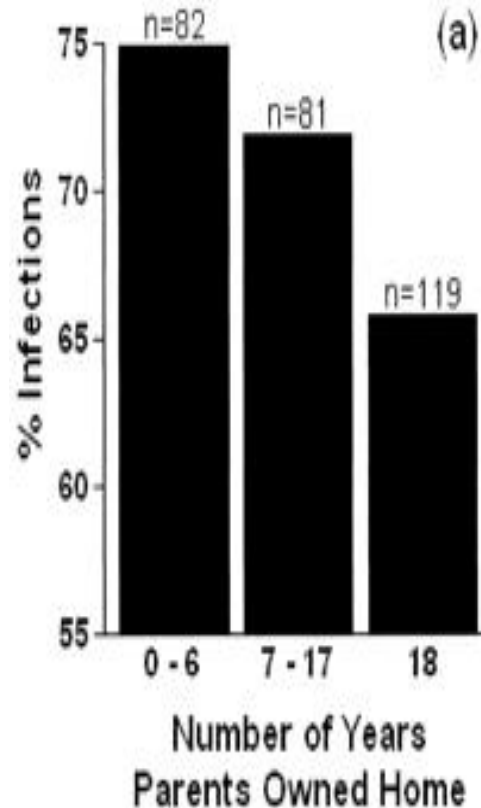
Data suggests more advantaged childhood social Position;
less likely to become infected with cold virus and among those infected less likely to develop full cold symptoms (Cohen et al., 1994)

- Inflammatory markers:

  - Relationship with social processes: good evidence for relationship with social position
  - Relationship with psychological processes: much work being done on depression/other psychological constructs
  - Relationship with behaviours: inflammatory markers may induce fatigue- influence on behaviours important
  - Relationship with disease: perhaps not aetiological to disease

Further reading

- **Dantzer & Kelley. 1989; 44: 1995-2008**
- **Eskanari et al., 2003; 5: 251-285**
  - Reviews of connections between neural and immune function

- **Yudkin, et al., Atherosclerosis. 2000;148:209-14**
  - Hypothesis paper outlining potential connections between obesity, stress, immune function

- **Webster Marketon JI**, **Glaser R**. Cell Immunol. 2008;252:16-26.
  - Recent review of psychological experiments by the Glasers, prolific psychoneuroimmunologists

'Practical' - comparing saliva sample and hair sample collection

Meena Kumari

CDT module 2: January 2020

'Collection of samples for the measurement of cortisol'

- Biomarker network protocol for saliva sample collection:

- http://gero.usc.edu/CBPH/network/resources/saliva.html

- Understanding Society protocol for hair sample collection

- https://www.understandingsociety.ac.uk/hair

Questions about these methods

- What do you think are the advantages of each of these collections?
- Discuss the difficulties of these collections
- Might there be differences by mode for this measure?
- Discuss the implications of these protocols on participants and participation

Comparing mode of sample collection

	N=330 households (N=500)	N=330 households (N=500)	N=330 households (N=500)	OUTCOME
Comparison 1	Nurse visit collection	Interviewer – leave	Participant led collection	1. Response rate: By age, sex, household composition, Social position 2. Total Cost per mode of invitation
Comparison 2	Whole blood draw (from adults) Blood spots (from adults) Hair sample collection (from all participants)	Blood spots(from adults) Hair sample collection (from all participants)	Blood spots(from adults) Hair sample collection (from all participants)	1. Comparison of biomarker measures by tissue 2. Response rate, error and bias by tissue and household composition 3. Biomarkers across adult age span 4. Steroids across the entire age range
Comparison 3	Feedback of blood results / No feedback	Feedback of blood results / No feedback	Feedback of blood results / No feedback	1. Influence of feedback on response rate. 2. Variation of influence of feedback by mode of collection and other factors such as household composition 3. Total cost per outcome

Preliminary results

	Nurse	Interviewer	Web
Blood pressure measurement before visit	33	28	42
Dried blood spot collection*	61	32	29
Full blood collection	60		
Hair sample*	55	31	24

Numbers represent percentage return by mode of sample collection

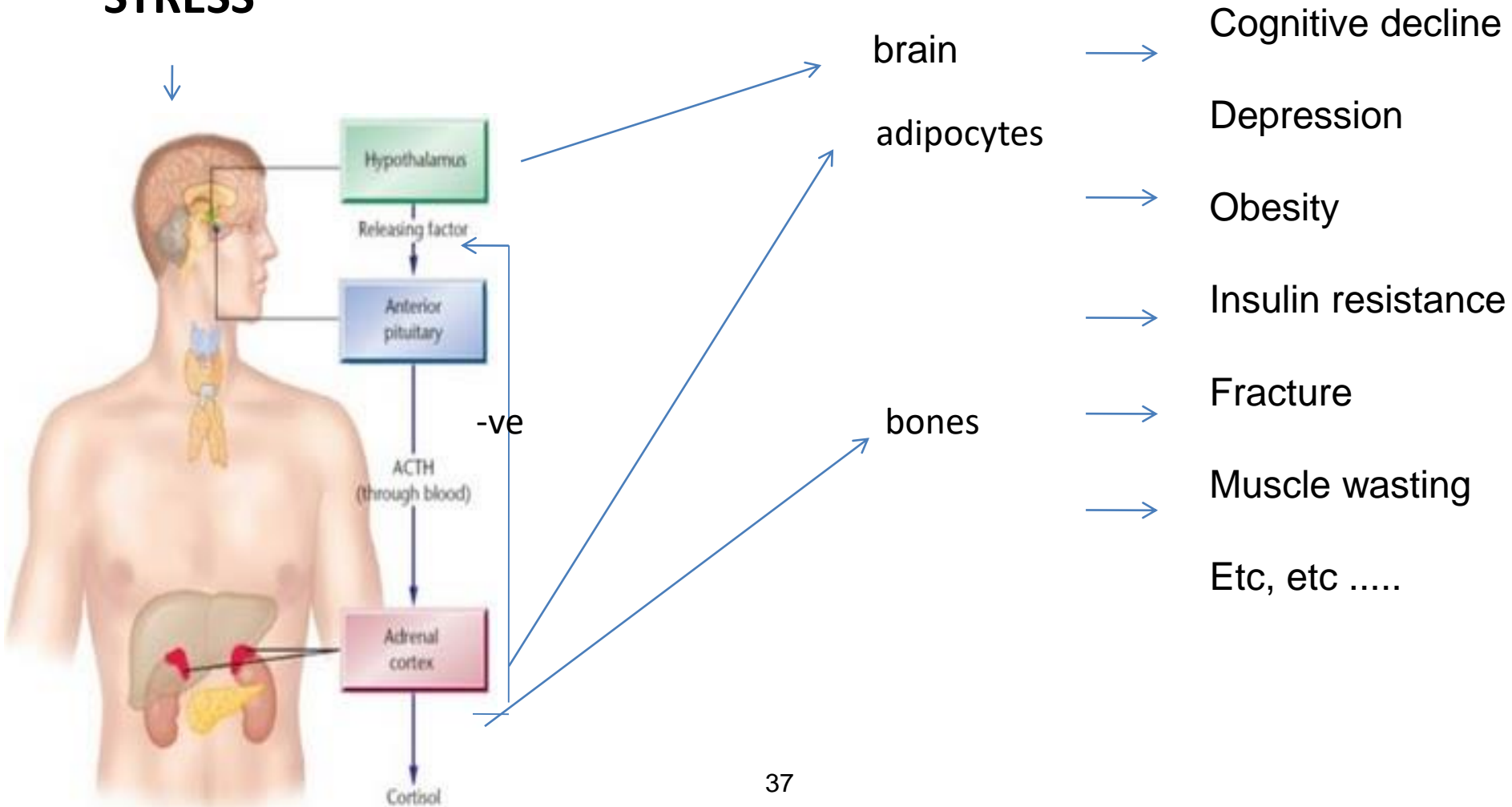* To December 2019 – another ~100 samples to be recorded

Neuroendocrine function

Meena Kumari

CDT module 2: January 2020

Neuroendocrine: the HPA axis

STRESS

↓

Hypothalamus

Releasing factor

Anterior pituitary

-ve

ACTH (through blood)

Adrenal cortex

Cortisol

brain → Cognitive decline

adipocytes

Depression

→ Obesity

→ Insulin resistance

bones → Fracture

→ Muscle wasting

Etc, etc .....

37

# Difficulties in measurement of the hypothalamic-pituitary-axis

- Component of patterns have different predictors?
  - Cortisol awakening response - 'state'
    - Both decreased and increased cortisol awakening response considered adverse

  - Rest of the day - 'trait' ?
    - 'flatter slopes' predictive of future deaths in a patient population

Collection of saliva samples in Whitehall II

- Collection of 6 saliva samples throughout the day (phase 7)
  - Waking*
  - +30mins*
  - +2h
  - +8h
  - +12h*
  - Bedtime*

*also collected in a sub-set of ELSA participants

Difficulties in analysis of the hypothalamic-pituitary-axis

Diurnal cortisol patterns

Cortisol awakening response

slope

Time since waking (h)
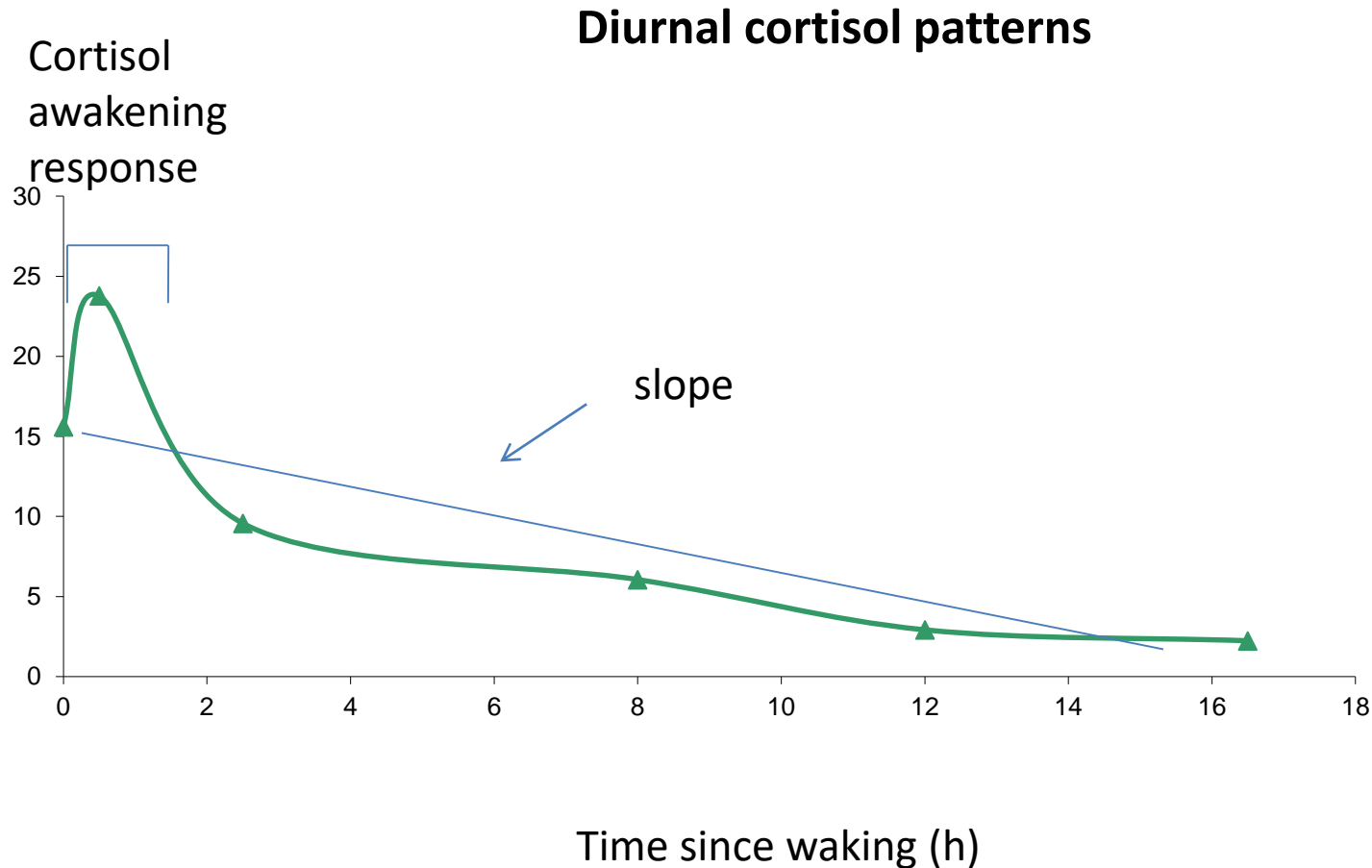
Cross sectional associations in Whitehall II

	Cortisol awakening response	Slope	
Smoking	Increased	Flatter	Badrick et al., 2008
Alcohol intake			Badrick et al., 2008
Fatigue	No association	Flatter	Kumari et al., 2009
'stress'	No association	Flatter	
'Happiness'			Steptoe et al., 2009
Sleep duration/disturbance	Increased	Flatter	Kumari et al., 2010
BMI/waist	No association	'U' shaped	Kumari et al., 2010
Diabetes	No association	Flatter	Hackett et al., 2014
Disadvantaged social positon	No association	Flatter	Kumari et al., 2010
'financial insecurity	No association	flatter	
Walking speed		flatter	Kumari et al., 2010

'Prospective' associations in Whitehall II

	Cortisol awakening response	Slope	
Maternal separation in early life	Increased	Flatter	Kumari et al., 2013
Future fatigue	No association	Flatter	Kumari et al., 2008
Diabetes and impaired fasting glucose	No association	flatter	Hackett et al., 2016
Mortality	No association	Flatter	Kumari et al., 2011

Do we need a new method to measure cortisol?

	serum	saliva	urine	Hair
Invasive	high	low	moderate	Low
Stressful	yes	possibly	possibly	No
Sample collection and Storage	Spinning and 'fridge	'fridge or freezing	'fridge or freezing	Room temp. stable for years
Time period	Single time point measure	Single point measure	12-24h integrated measure	Months integrated measure
Data analysis	Multiple data points required	Multiple data points required	Single point usable	Single point usable

Response rates

	Number of participants asked	%agreed	% response
Phase 7	4967	96	91
Phase 9	6044	99	84

Phase 9 – 84% independently of participation in cortisol protocol at phase 7

Solution: collection of cortisol in hair

- Why useful?
  - Easy to collect
  - Data are easier to handle than diurnal pattern
  - Chronic exposure to cortisol
  - Not influenced by acute stress vagaries

- Why not?
  - Doesn't give us the same information as diurnal pattern

Potential difficulties

- influence of hair characteristics

  hair colour

  frequency of washing

  hair treatments

- no hair!

Sample collection at phase 11: what happened?

- 77% of participants that came to clinic resulted in a sample with a cortisol measurement. Why?
  - 20% of men had insufficient hair..
- *Compare these response rates to those seen in Understanding Society? What's happening?*

Do data have face validity?

Associations with adiposity, type 2 diabetes (Abell et al., 2016); depressive symptoms (Abell et al., under review). Cognitive function analysis ongoing..

More complex outcomes

- Combining biomarkers
  - Metabolic syndrome
  - combination of adiposity, lipids, blood pressure and glycaemia

  - Allostatic load
  - markers of multi-physiological systems

Allostatic load: what is it and why is it interesting to social scientists

Allostasis: the physiological process of maintaining stability (homeostasis) through adaptation, by releasing stress hormones (cortisol, epinephrine).

Short term: essential for body adaptation/survival.

Long term: damage effects on the body, accelerate disease, weaken immune system, cause of disease.

Allostatic Load (AL): term coined by McEwen and Stellar in 1993 (Archives of internal medicine 153 (18): 2093–101)

"the wear and tear on the body" which accumulates over time, after acute or chronic stress.
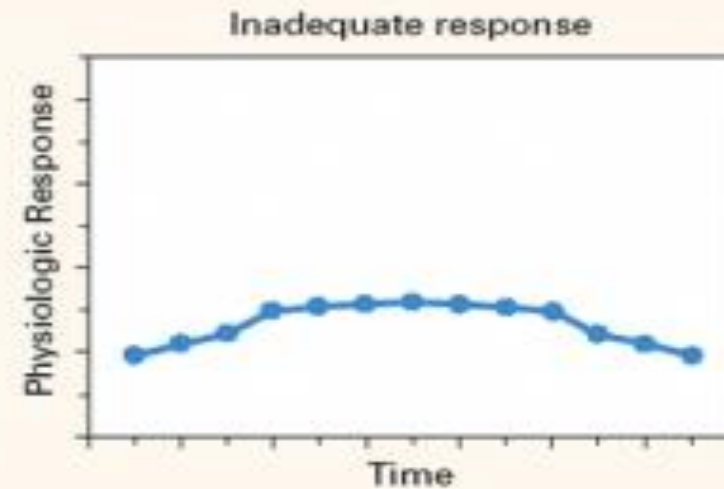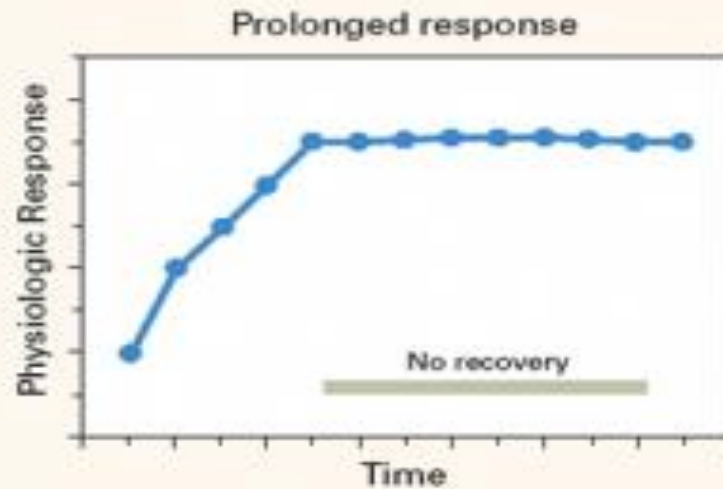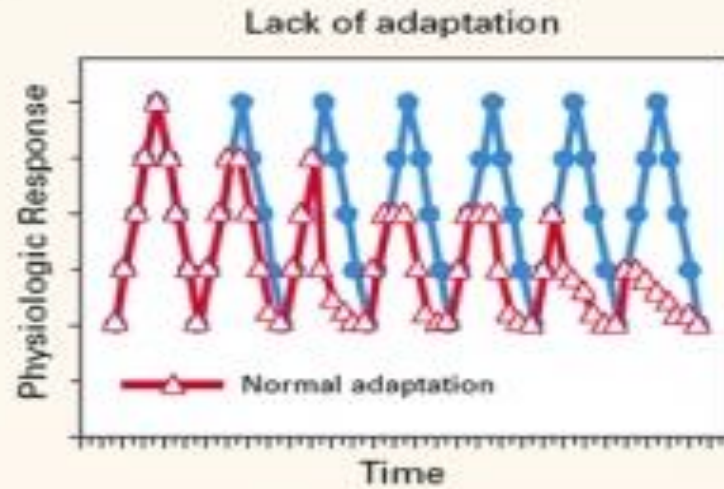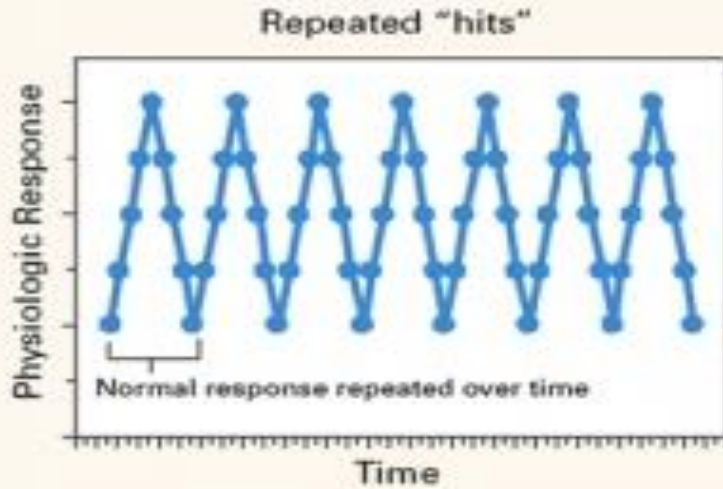
AL is generally measured through a composite index of indicators (biomarkers) of cumulative stress on several physiological systems.
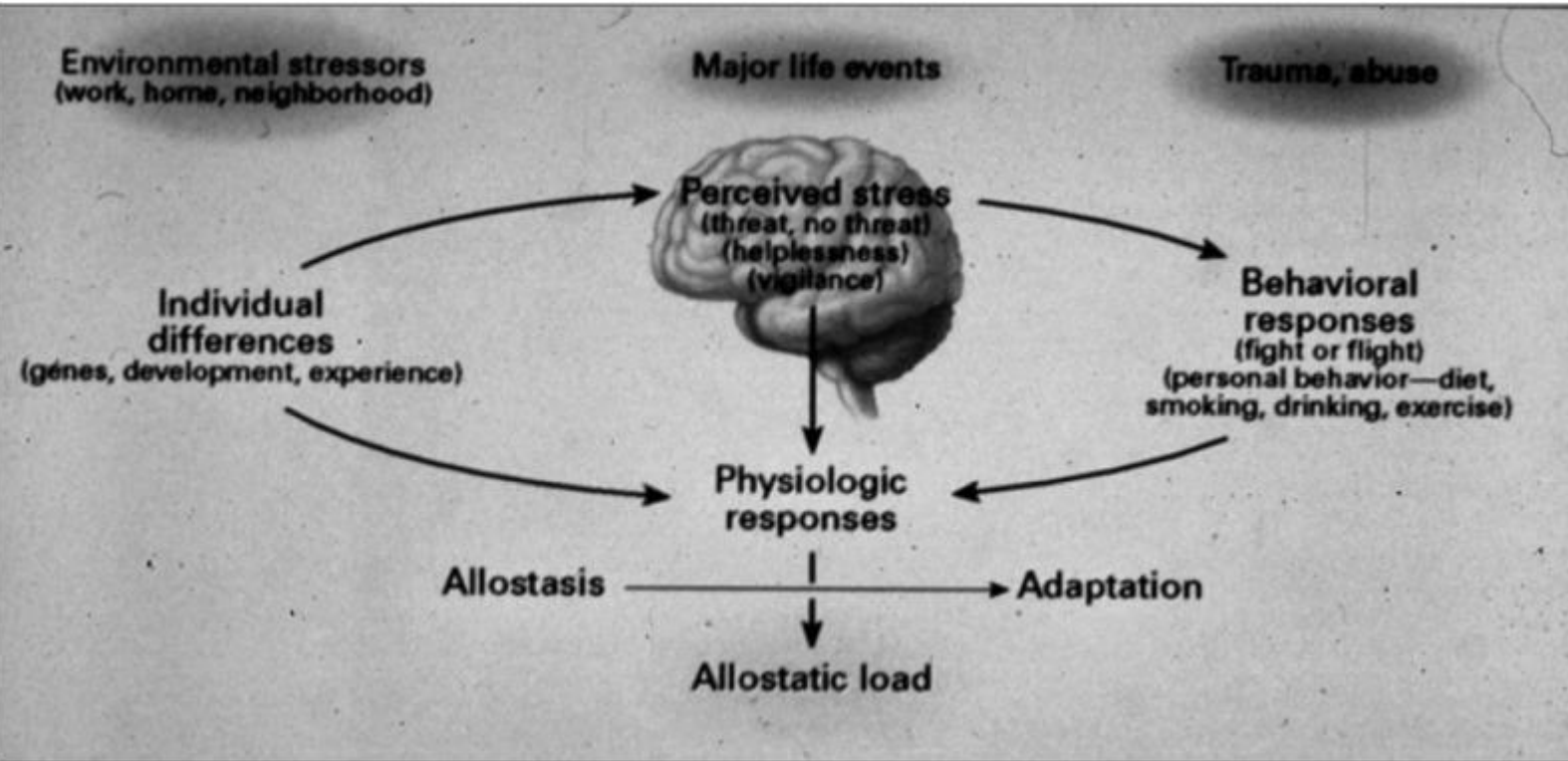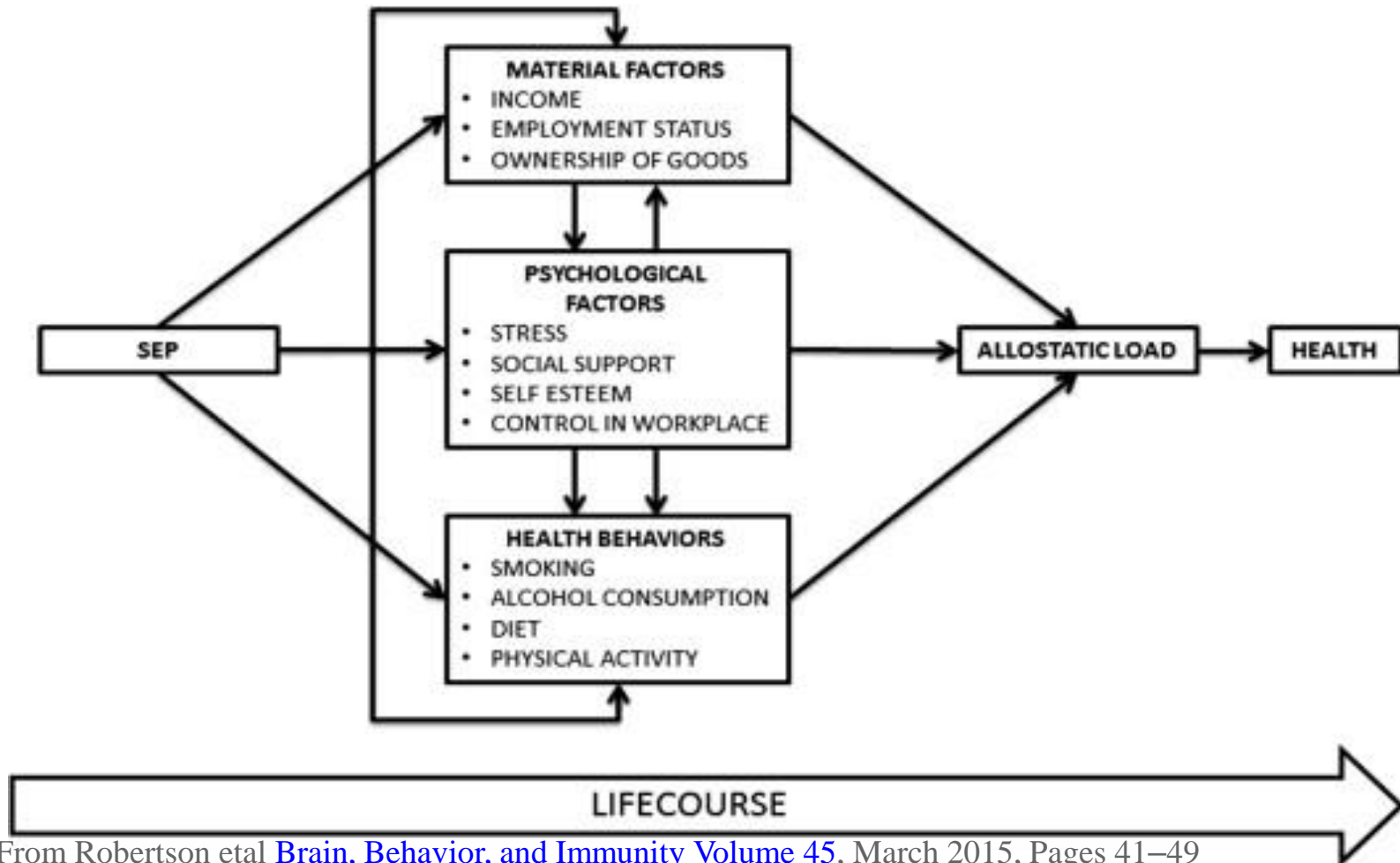
Allostatic load

Mcewen PNAS; 2012

Allostatic load

Important tool for understanding the association of socioeconomic status with health and mortality (*National Research Council/2001*)

Operationalising Allostatic load

Primary mediators

Secondary outcomes

Tertiary outcomes

Cortisol
Adrenaline
noradrenaline
DHEAs

Waist-hip ratio
Blood pressure
HBA1c
Lipids
Fibrinogen
"immune function"

Cardiovascular disease
Physical capacity
Cognitive decline

↓

Primary effects

Epigenetic changes
Gene expression
Protein-protein interactions

From McEwen and Seeman:
Allostatic load notebook 2009

Operationalising 'allostatic load' in the literature

- No convention
  - Either in composition (which biomarkers?)
  - Or in method of data combination

- Simple addition
  - 'adverse' biomarker levels
    - clinical cutpoints
    - Population specific cutpoints

- Factor analysis

- Canonical correlation

Our approach: Factor Analysis

- Use factor analysis to identify common structure (latent) among AL-related biomarkers.

- AL theory: handful of factors will describe variation among large number of biomarkers
  - A factor is a combination of biomarkers.
  - Interpret factor by its 'loadings'
  - Loading gives biomarker's contribution and "meaning" to factor
  - Generally, significant contribution only if loading > 0.5
  - Factors can be correlated (oblique rotation)

- Does same structure emerge for all studies?

Factor Analysis Steps

- Step 1: Selecting and Measuring a set of variables in a given domain i.e., range of biomarkers

- Step 2: correlation matrix

- Step 3: Factor Extraction

- Step 4: Factor Rotation to increase interpretability

- Step 5: Interpretation

- Further Steps: Validation and Reliability of the measures

FA in CLOSER datasets

Focus on datasets that contain Neuro-Endocrine (NE) markers, (primary mediators):

- NSHD: **Cortisol (saliva), DHEAS, IGF-1,**

- NCDS: **Cortisol (saliva), IGF-1**

- UKHLS: **DHEAS, IGF-1**

- HCS: **Cortisol (blood and saliva)**

- ELSA, waves 4 and 6 (not in CLOSER): **DHEAS , IGF-1**

Interpretation of Factors:

Metabolic

- Factor 1: BMI, HDL, Triglycerides, waist, Insulin
- Factor 2: Total cholesterol, LDL
- Factor 3: HbA1c, Glucose

Cardiovascular

- Factor 4: Blood pressure (Diastolic and Systolic)

Inflammatory

- Factor 5: IL-6, CRP, (Albumin)

Neuro-Endocrine

- Factor 6: Cortisol (CAR), DHEAs, IGF-1

Kidney/Liver

- Factor 7: Urea, Creatinine
- Factor 8: GGT, ALT, AST

Factors by Study*+

Factors	Study					
	ELSA		HCS	NCDS	NSHD	UKHLS
	Wave 4	Wave 6				
Metabolic	BMI	BMI	BMI	BMI	BMI	BMI
	Waist Circumference	Waist Circumference	Waist Circumference	Waist Circumference	Abdominal Circumference	Waist Circumference
	HDL	HDL	Triglycerides	Triglycerides	Triglycerides	Triglycerides
	Triglycerides	Triglycerides	Creatinine	HDL	HDL	HDL
	HbA1c	CRP	Proinsulin	t-PA	SHBG	
	CRP		Insulin			
Blood Pressure	Pulse	Pulse	SBP	SBP	SBP	SBP
		Mean Arterial Pressure				
	Mean Arterial Pressure		DBP	DBP	DBP	DBP
			Lipoprotein (a)			Pulse
Lipid	Total Cholesterol	Total Cholesterol	Total Cholesterol	Total Cholesterol	Total Cholesterol	Total Cholesterol
	LDL	LDL	Triglycerides	LDL	LDL	Triglycerides
			LDL			LDL
			APO-B			
			HDL			
			APO-A			
Inflammatory	Fibrinogen	Fibrinogen	Pulse	Pulse		CRP
	CRP	WBC	CRP	CRP		Fibrinogen
		CRP	Fibrinogen	Fibrinogen		
			Lipoprotein (a)	vWF		
Glucose Metabolism	DHEAS	HDL		HbA1c		
	HbA1c	Triglycerides		IGE		
	Triglycerides	IGF-1				
		HbA1c				
		Haemoglobin				
		WBC				
Iron Storage	Ferritin	Ferritin				Pulse
	Haemoglobin	HbA1c				Ferritin
	HbA1c	Haemoglobin				IGF-1
	MCH	MCH				Haemoglobin
Neuroendocrine			Triglycerides	IGF-1	DHEAS	Albumin
				Cortisol awakening response		
			Creatinine		Testosterone	DHEAS
			Cortisol		HDL	HbA1c
			Glucose		IGFBP-3	Haemoglobin
				IGF-1	IGF-1	IGF-1

Summary and conclusion

- Extracted Latent factors make sense in terms of theory/expected finding

  » Metabolic (more than one)

  » Cardiovascular

  » Inflammatory

  » NE

  » Kidney/liver (more than one)

- But factors are not the same across studies because studies have each measures slightly different biomarkers:

  – Vary by age?

  – Vary by social position

Adjusted odds of high AL per educational attainment, relative to having a degree
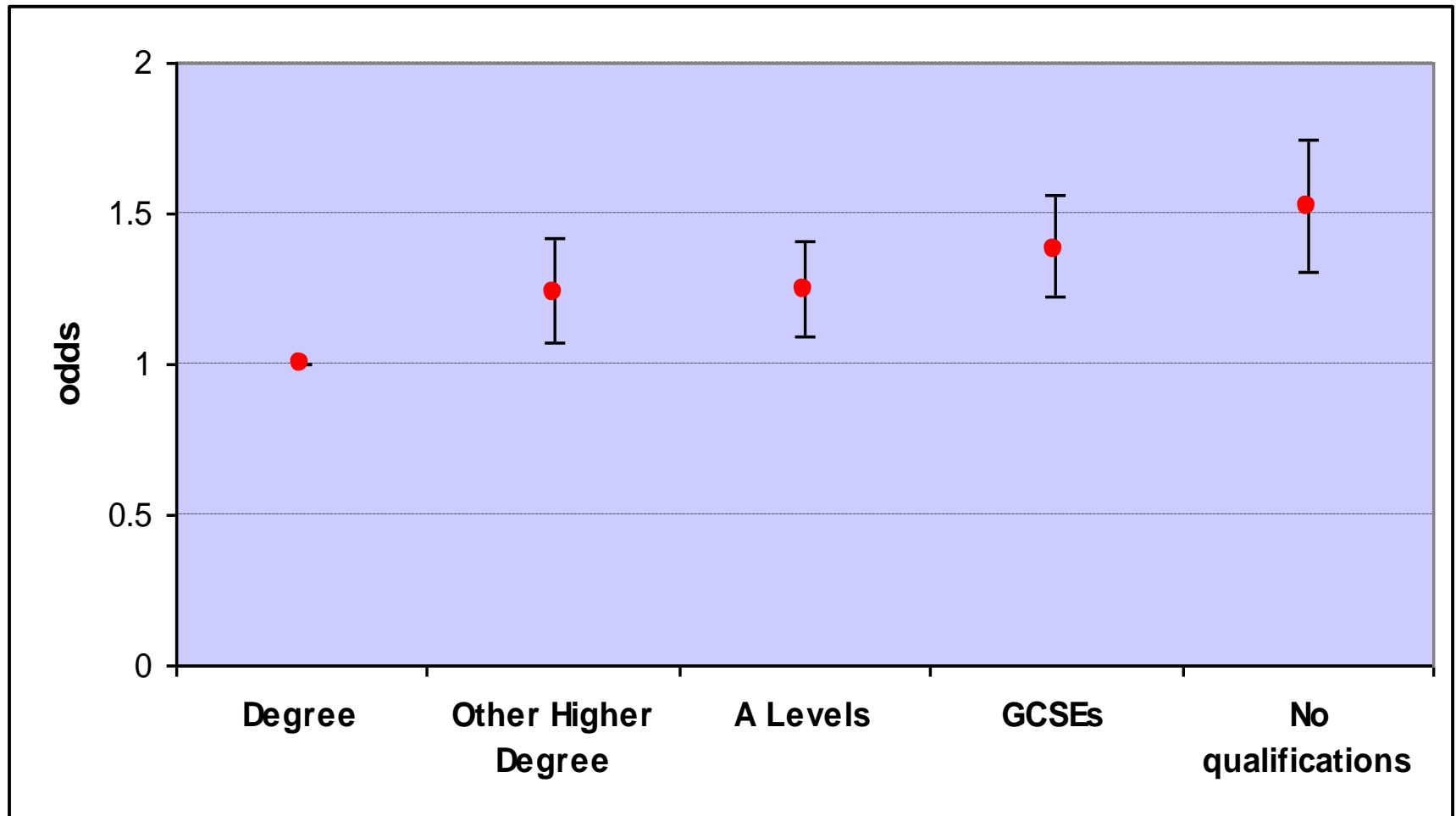
Educational attainment used as an example here….

Discussion

- Individual pathways vs. combinatorial approaches ('common soil')

- Is it useful to combine biomarkers in a biosocial framework?

You may wish to reflect on:

- Biological pathways: do the biomarkers available in secondary data resources optimally capture the pathways you are interested in?

- Who have you collected these biomarkers from?
  - Does it matter?

Data sources: biomarkers

- UKDA
  - *Understanding Society*
  - English Longitudinal Study of Ageing
  - Health Survey for England
  - Scottish Health surveys
  - NCDS (1958 birth cohort)
  - BCS70 (1970 birth cohort)*

*forthcoming

Data sources: genetic and genomic data

- DNA
  - *Understanding Society*
  - English Longitudinal Study of Ageing
  - NCDS (1958 birth cohort)
  - BCS70 (1970 birth cohort)*
  - Millenium cohort study*
  - ALSPAC (children of the '90s)
  - Hertfordshire Cohort Study
  - NSHD (1946 British birth cohort)
  - Southampton Women's Survey

} Metadac.ac.uk

} Closer.ac.uk

- Epigenetic
  - *Understanding Society*
  - NCDS (1958 birth cohort)*
  - BCS70 (1970 birth cohort)*

Data information

- CLOSER: catalogue of the biomarker data

https://www.closer.ac.uk/wp-content/uploads/A-guide-to-the-biomarker-data-in-the-CLOSER-studies-FINAL.compressed.pdf

Over 50 biomarkers in over 50,000 participants

- *Understanding Society*

https://www.understandingsociety.ac.uk/documentation/health-assessment

21 biomarkers in 13,000 participants

After the break

- What do we need to think about if we want to collect data with 'the watch' ?