

ALKAPTONURIA

Pathophysiology
Signs and symptoms
Old treatment
Prognosis
New treatment?

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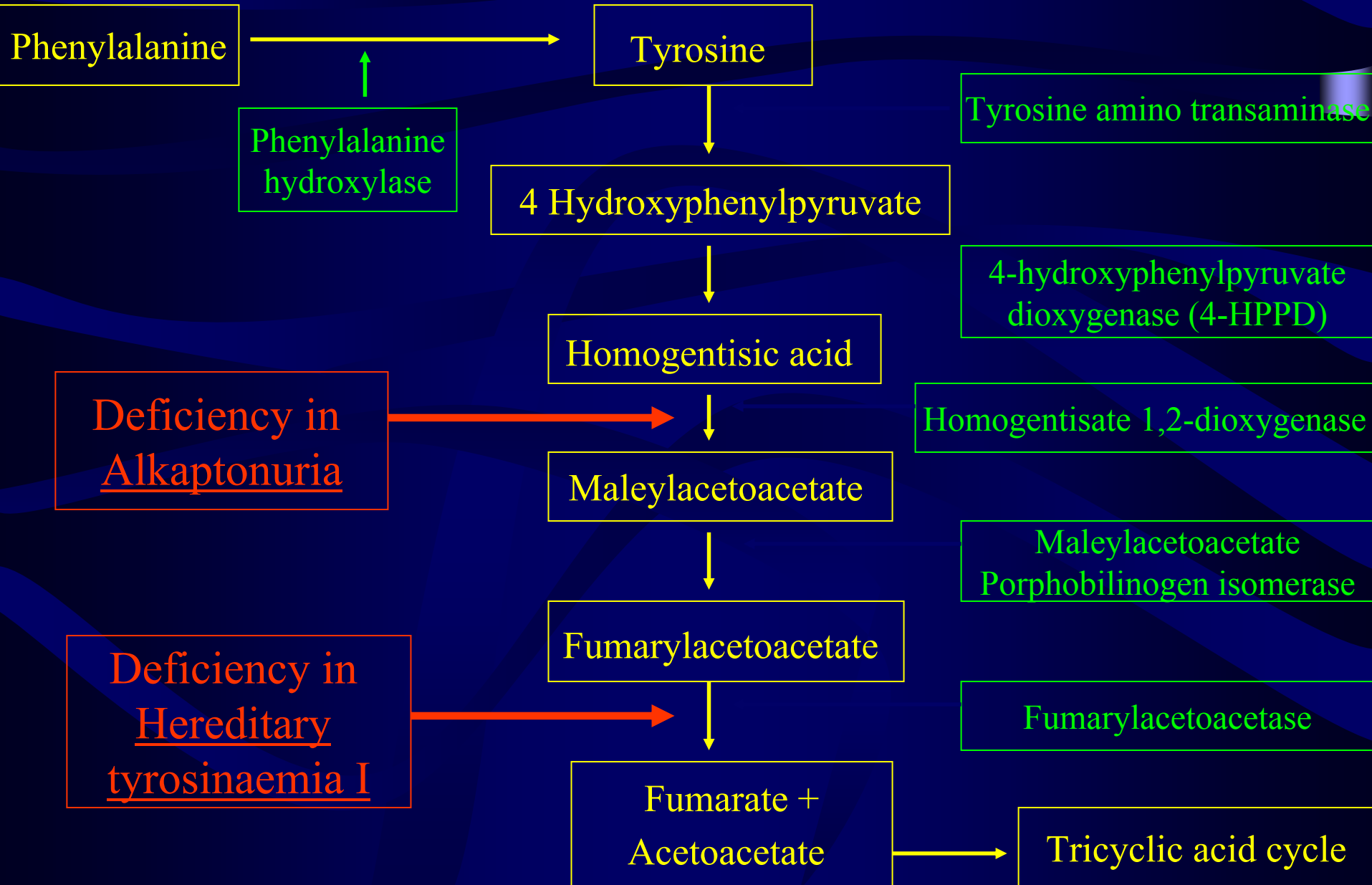
Parts of the presentation

- ❑ Background of the disease
 - Biochemical pathophysiology
 - Pathology
 - Genetics
- ❑ Signs and symptoms
- ❑ Complications and prognosis
- ❑ The Dutch survey
- ❑ Current treatment
- ❑ New treatment
 - How to test effectivity, safety and toxicity in orphan drugs
 - What about NTBC?
- ❑ Research & Development; outcome analysis
- ❑ Conclusions

Background of the disease

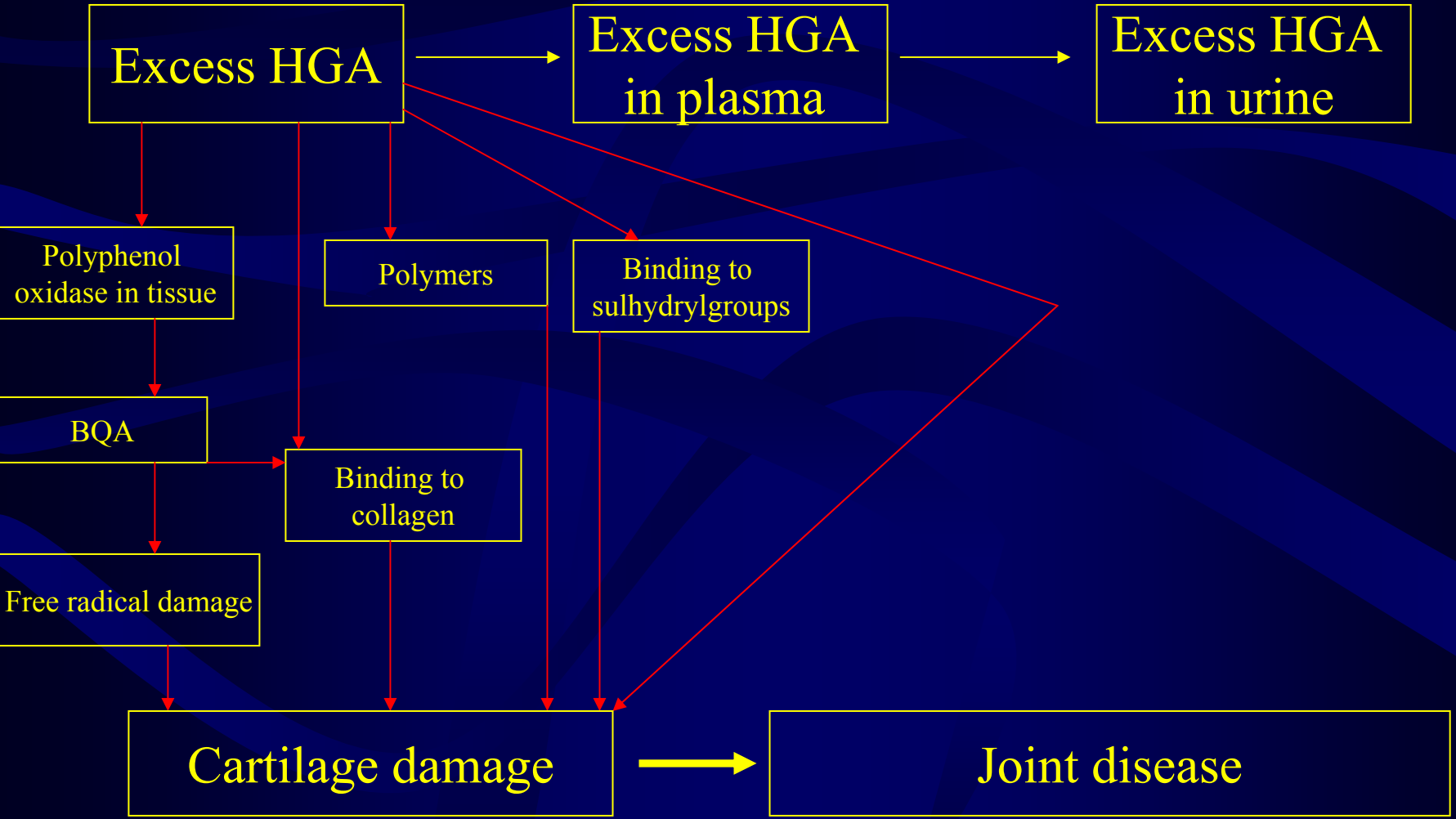
- ❑ Deficiency of the enzyme homogentisic acid oxidase
- ❑ Accumulation of homogentisic acid in blood, tissue and urine
- ❑ Rest activity can vary from person to person

Biochemical pathway



Genetic defect

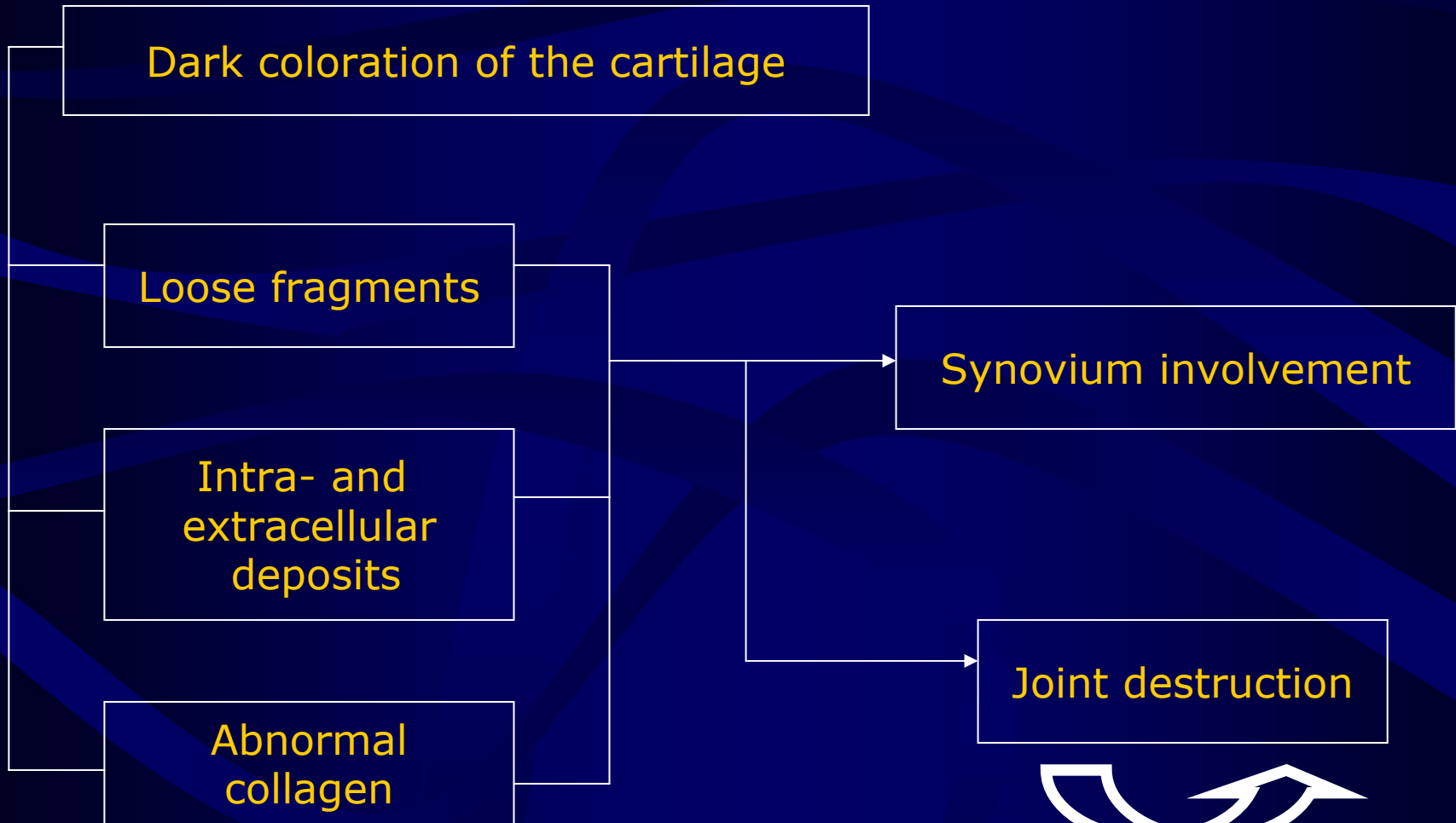
Pathophysiologic pathway



Genetics

- ❑ Autosomal recessive disease
- ❑ Many DNA-mutations
- ❑ Detected hot spots:
 - a valley in Slovakia
 - Dominican republic
 - geographically and socially secluded area
 - non-paternity
- ❑ Prevalence: 1/500.000 to 1/1.000.000
 - France (80 million): 80-160 patients
 - Les Pays Bas (16 million): 16-32 patients
 - European union (350 million): 350-700 patients

Pathology

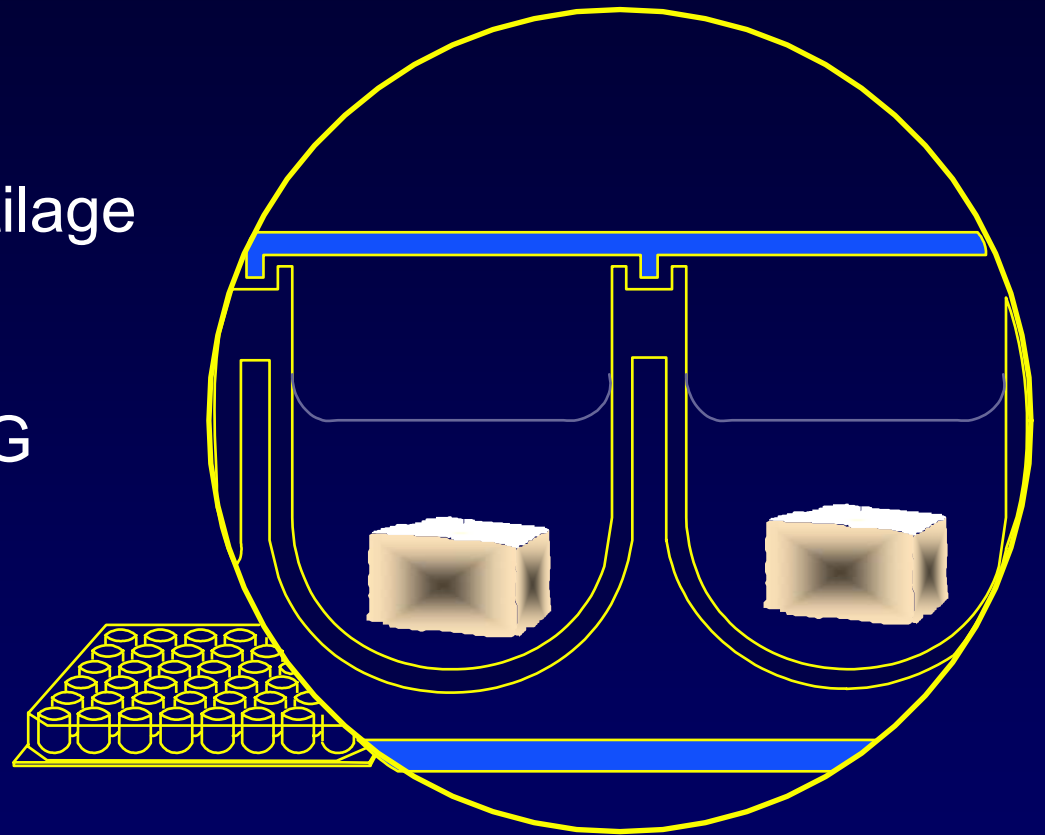


Culture system

Human articular cartilage

4 days culture

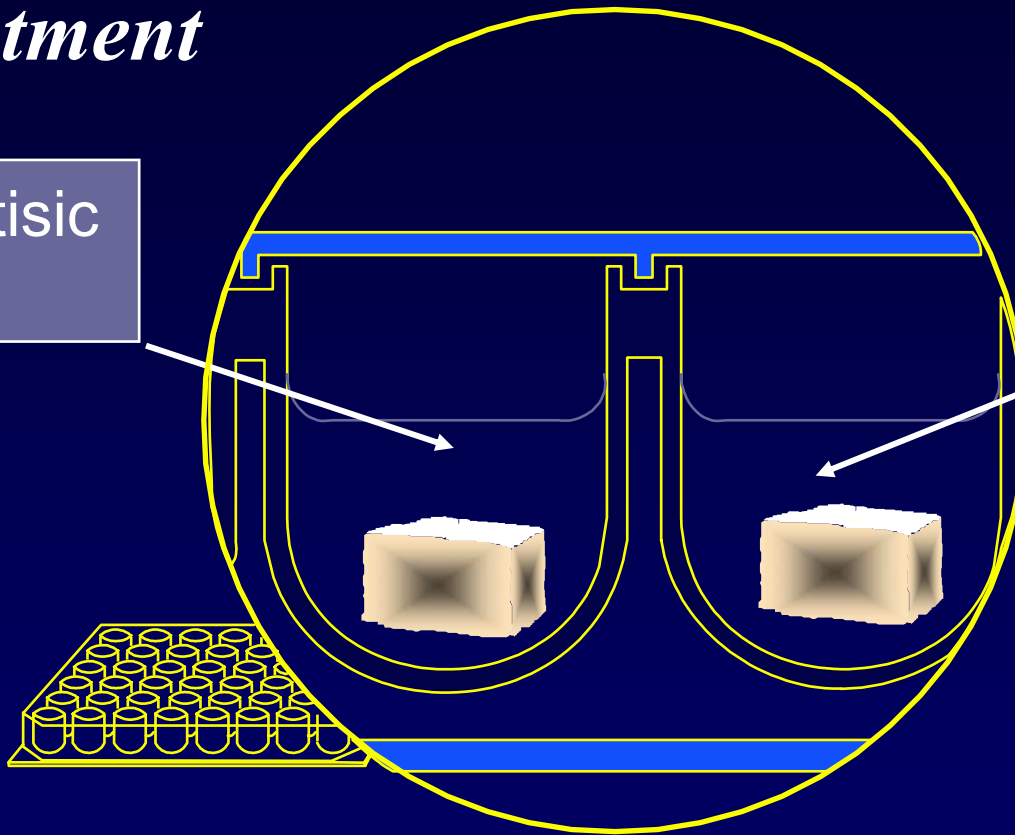
DMEM + AHS + PSG



Treatment

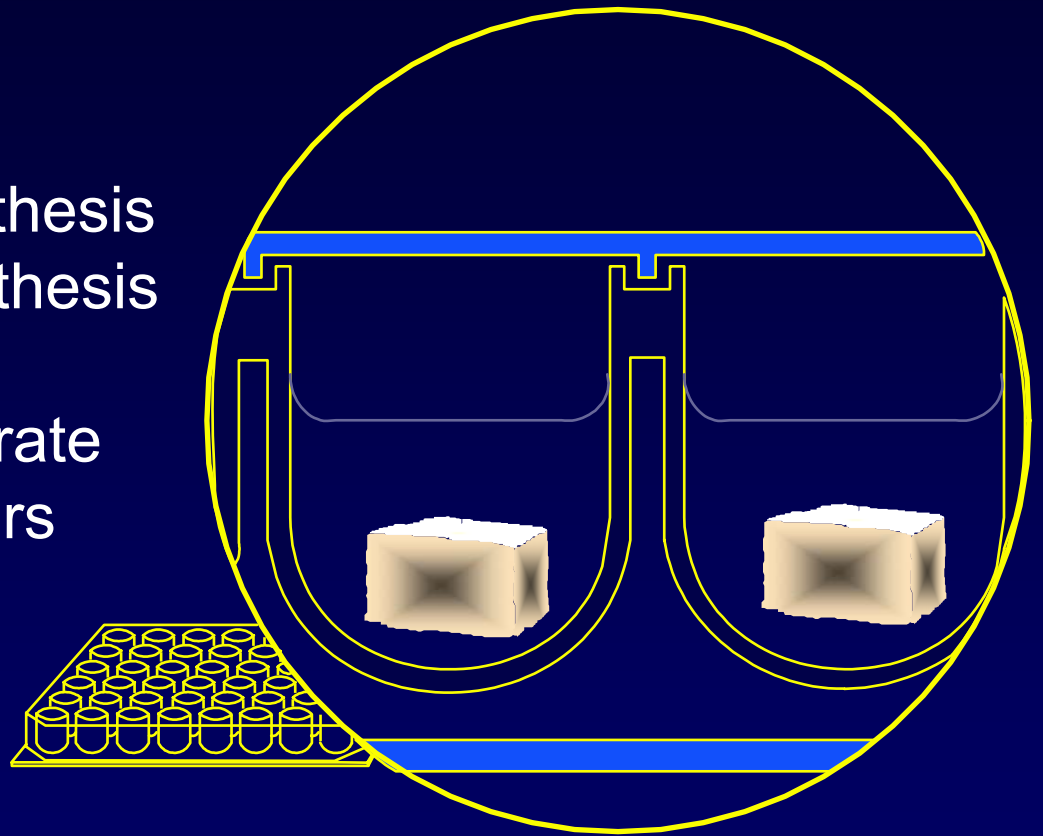
Homogentisic acid

Homogentisic acid and vit C

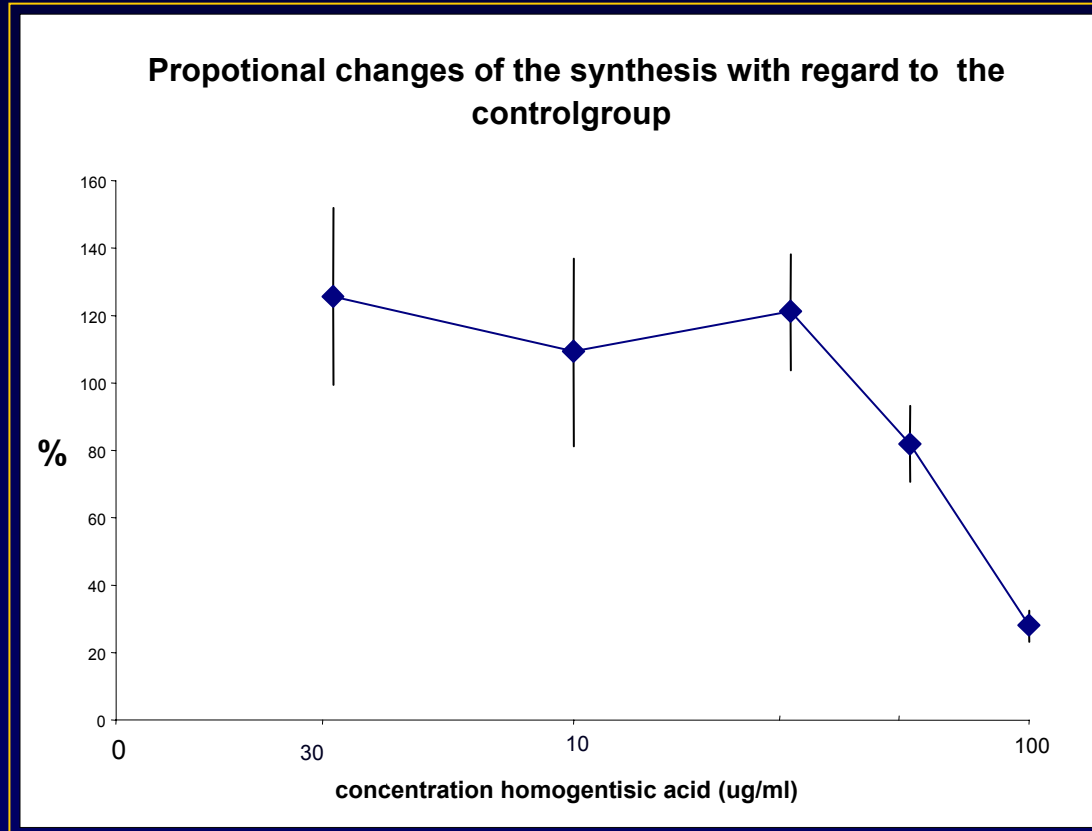


Analysis

Cartilage matrix synthesis
by proteoglycan synthesis
measured by
 $^{35}\text{SO}_4$ incorporation rate
during the last 4 hours
of culture



Results



Signs, symptoms and complications (1)

❑ Discoloration of fluids and tissues

- Discoloration urine
- Discoloration of sclerae
- Discoloration of cerumen
- Discoloration of sweat
- Discoloration of cartilage

❑ Joint disease at an early age

- pain
- limited mobility
- inflammatory episodes

Signs, symptoms and complications (2)

- ❑ Renal calculi
- ❑ Prostate calculi
- ❑ Cardiac valve abnormalities
- ❑ Conduction hearing loss
- ❑ Rupture of the Achilles tendon

Clinical evolution of alkaptonuria

A life's time

Urine

Sclerae

Urinary Calculi

Joints

Valvular disease

The Dutch survey

Research questions:

- What is the prevalence
- What are the clinical signs and symptoms
- What about radiographic evaluation of the skeletal system
- What about ultrasound evaluation of cardiac valves and function
- What about the current and previous therapy
- First estimation of incidence of complications
- Start of a (larger) follow-up study

The Dutch survey

Recruitment of patients:

- Contacting known professionals dealing with inborn errors of metabolism
- Contacting laboratories
- Contacting siblings and other family members
- Chance (1)

The Dutch survey

Research protocol:

- Written questionnaire completed by the patient
- Physical examination
- Radiographic evaluation:
 - spine
 - hip
 - knee
 - shoulder
- Ultrasound evaluation of cardiac valves and function

The Dutch survey

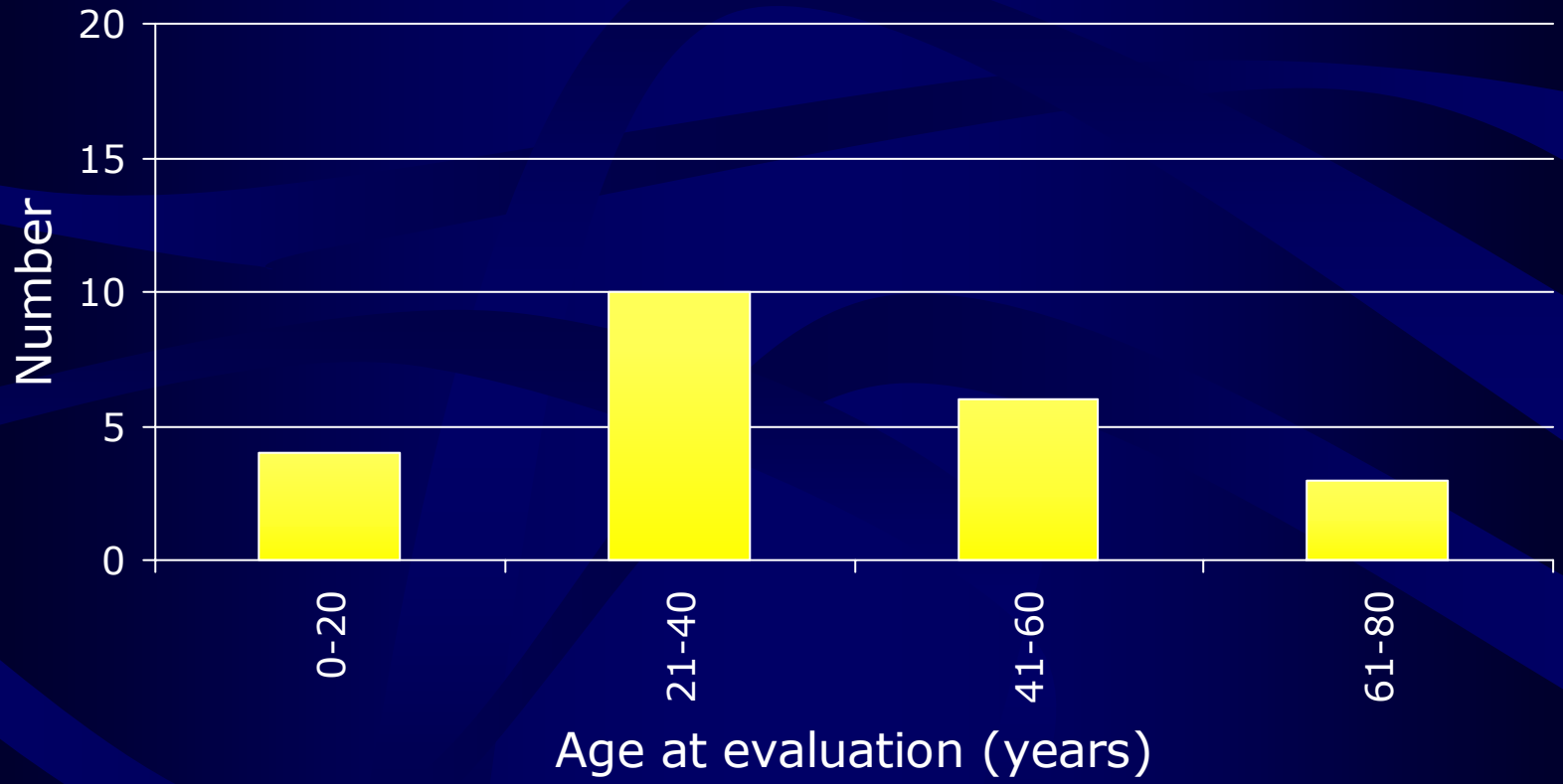
Results:

- Number of patients: 40 (32 adults; 23 presented)
- Prevalence: 1/400.000 subjects
- Sex (n,%): 18/5 (78/22)
- Age (years): 40 (16-72)
- Early-diagnosed (n,%): 14 (61)
- Late-diagnosed (n,%): 9 (39)
- Joint replacement (n): 3
- Spinal surgery (n): 1
- Cardio-vascular disease (n): 1

Age at diagnosis



Age at evaluation



The Dutch survey

Results:

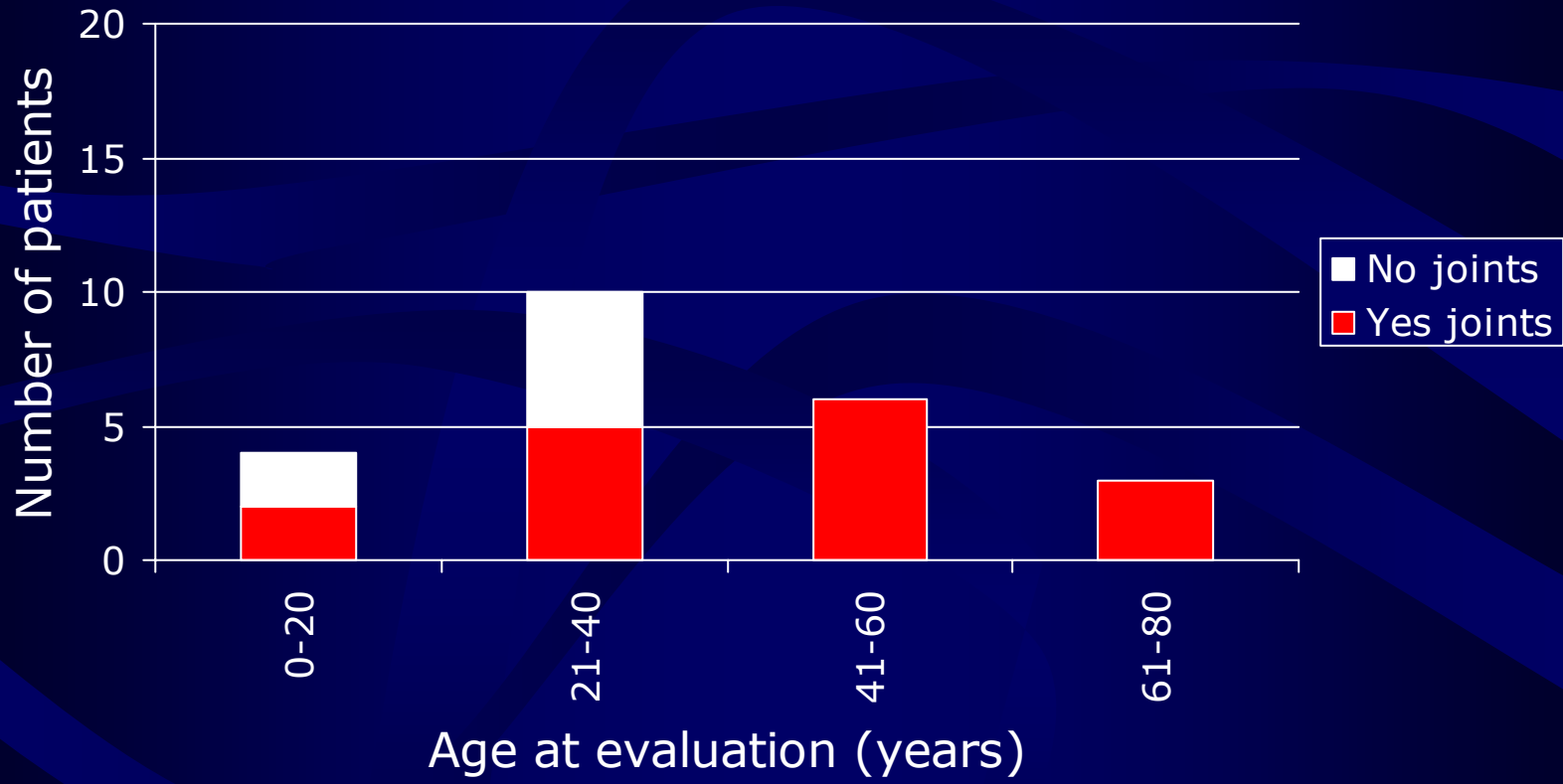
- Spinal pain: 16/23 patients 70%
- Joint pain: 15/23 patients 65%
 - shoulder 8/23 patients 35%
 - hip 11/23 patients 48%
 - knee 11/23 patients 48%

The Dutch survey

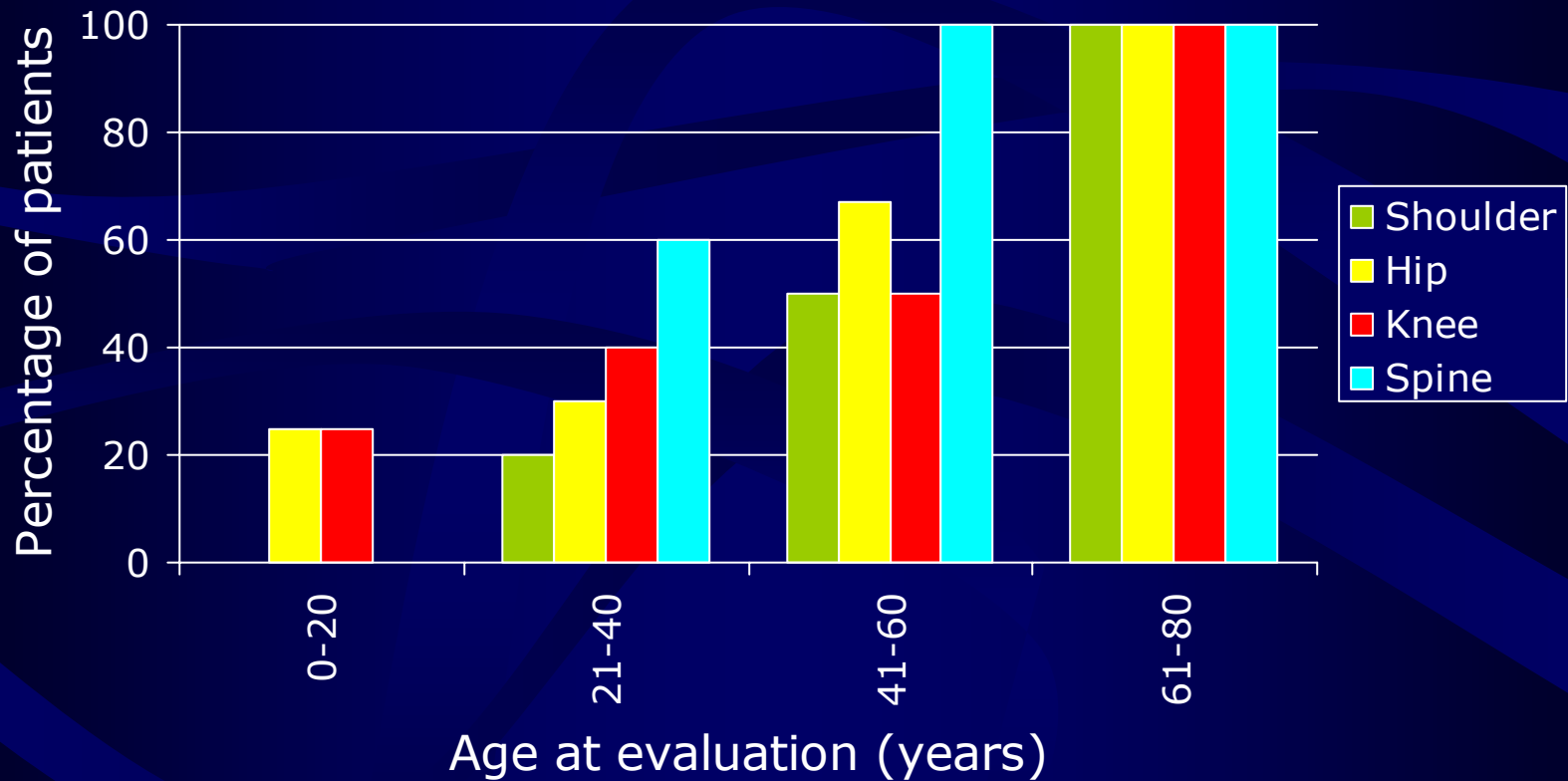
Results:

- 8 patients stopped working or changed job because of the disease
- No patients with renal calculi
- 1 patient with prostate calculi
- 2 patients with an Achilles tendon rupture
- 5 patients with mild hearing loss
- 1 patient with a valve replacement
- No patients with coronary heart disease

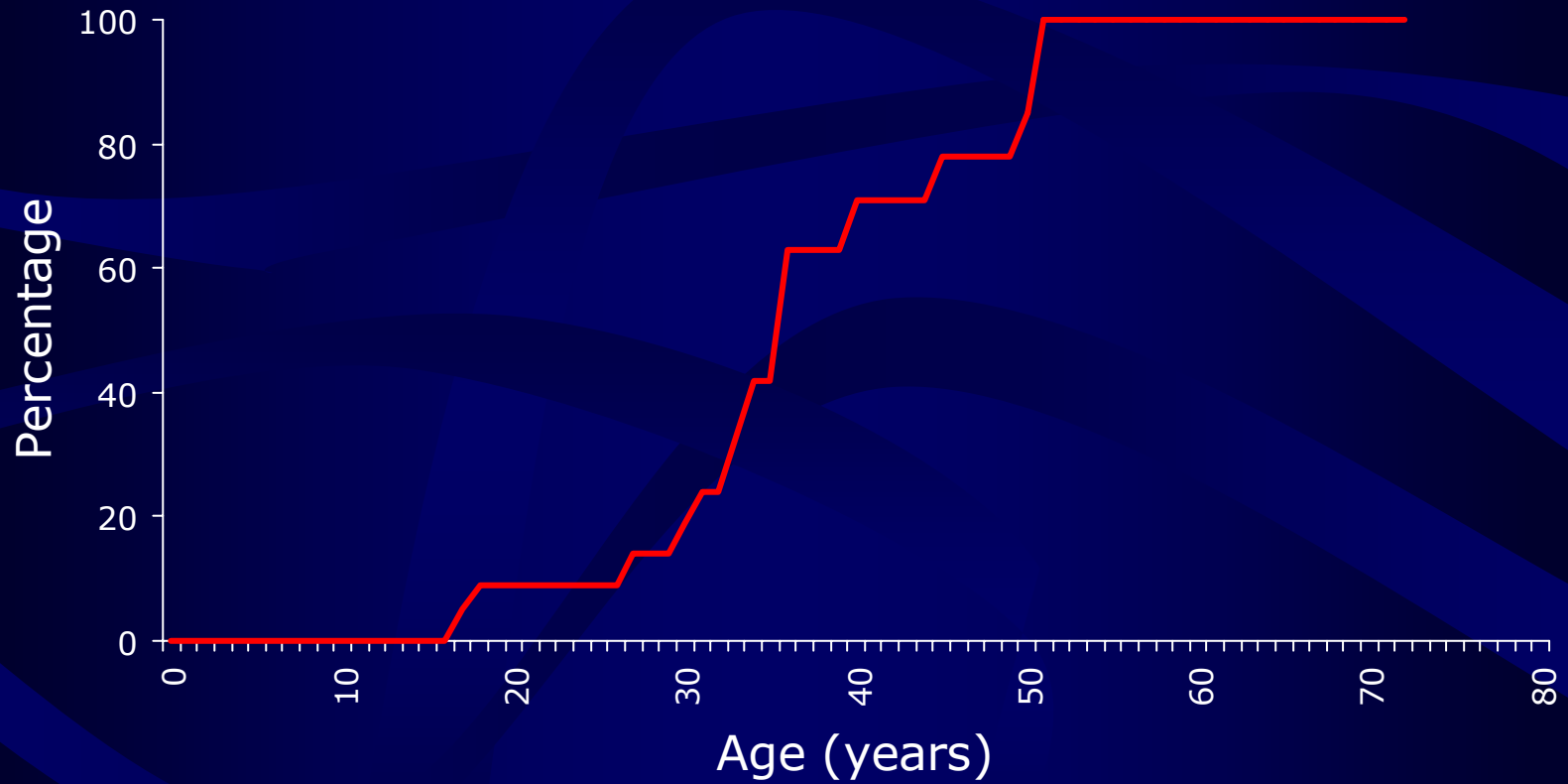
Painful joint(s)



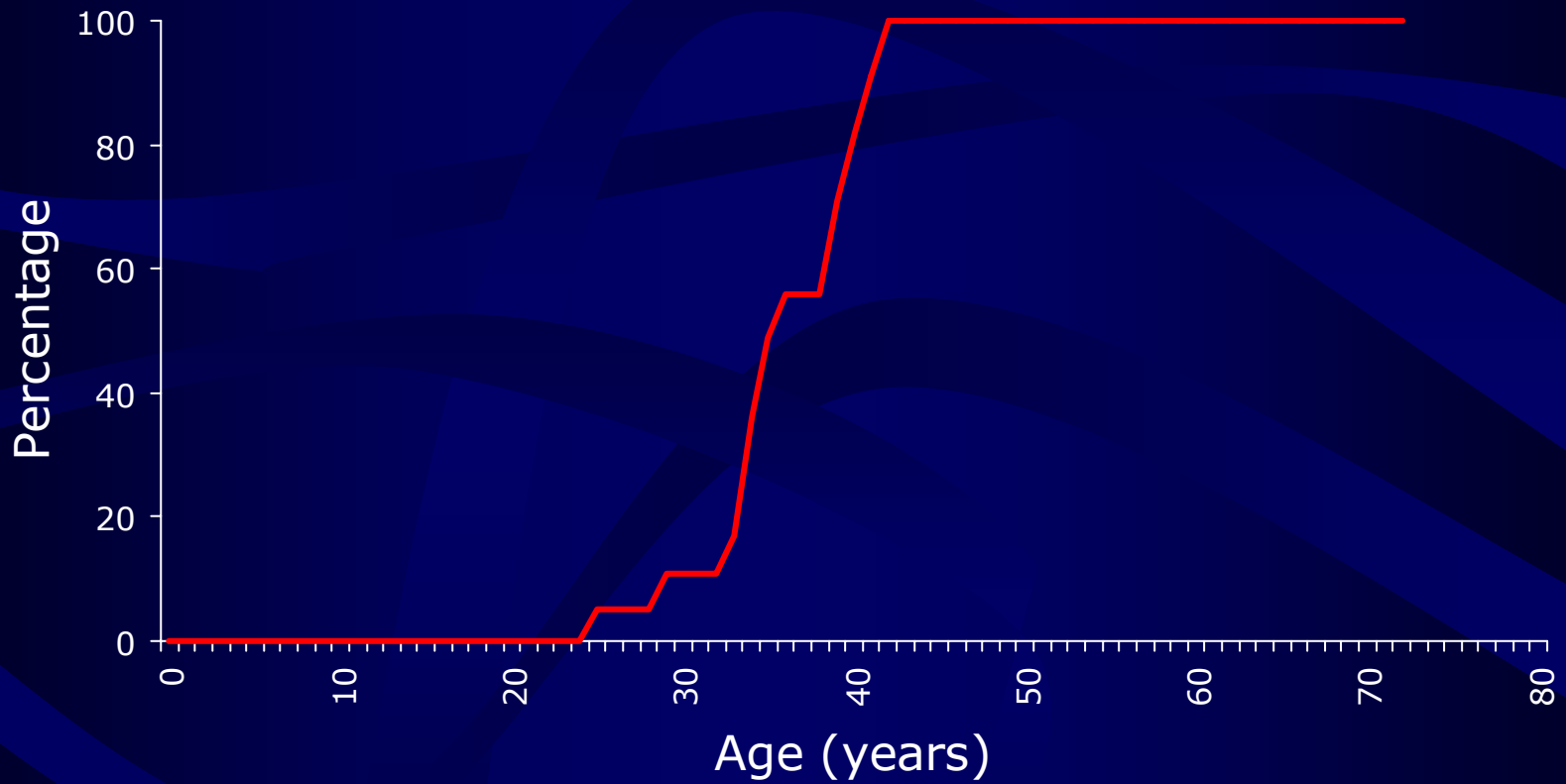
Pain according to joint involved



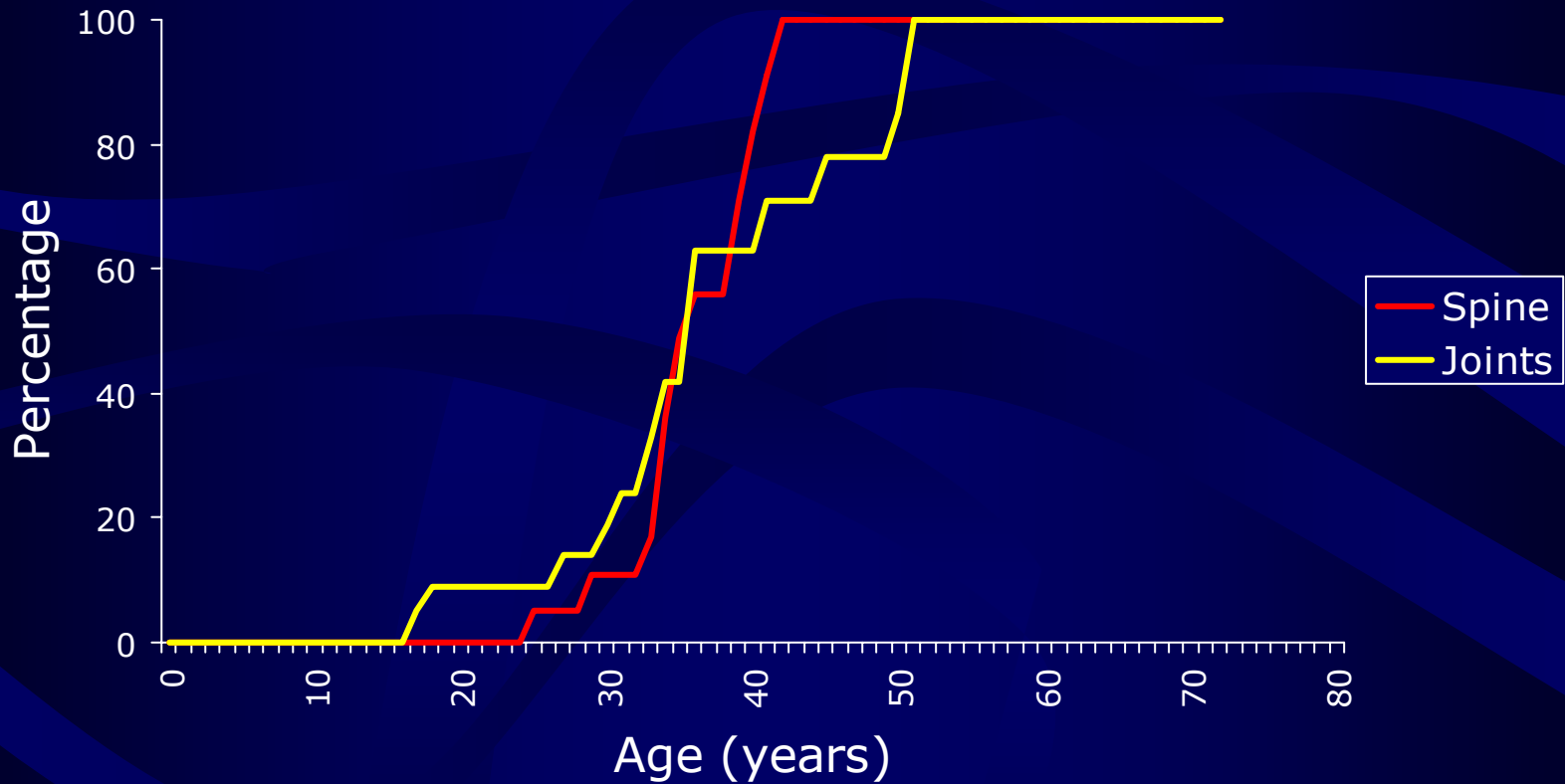
First symptom large joints



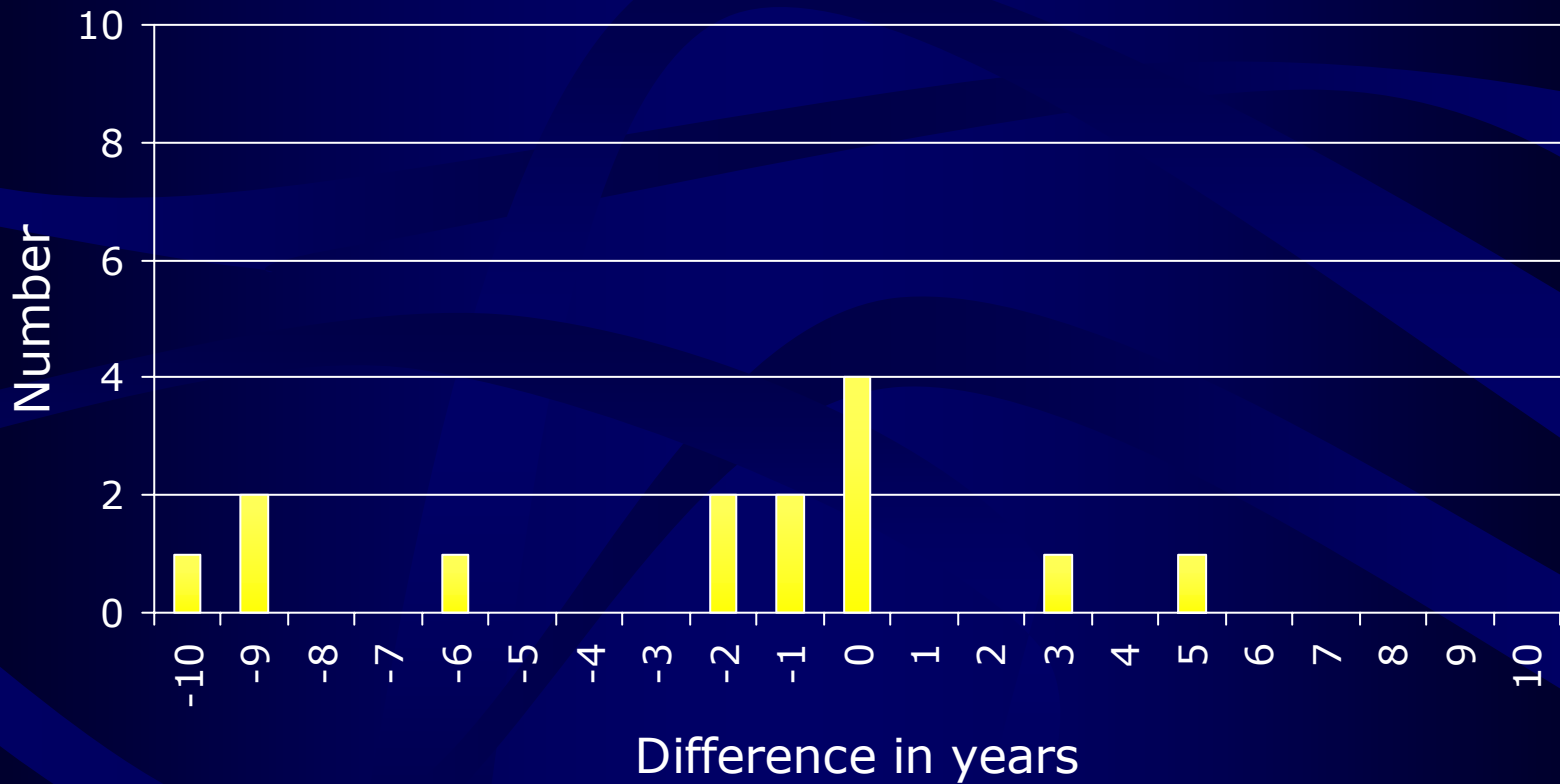
First symptom spine



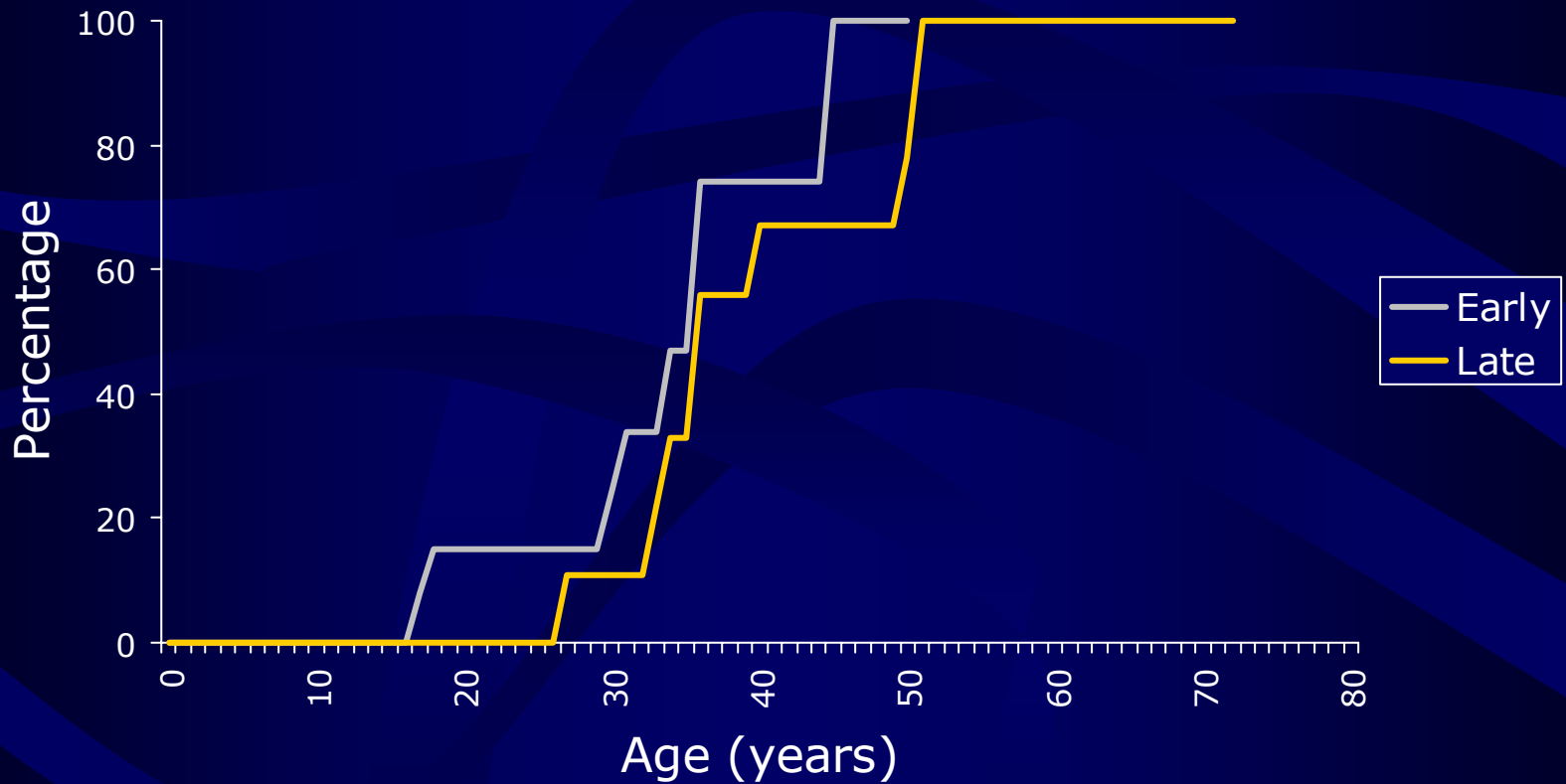
First symptom joints & spine



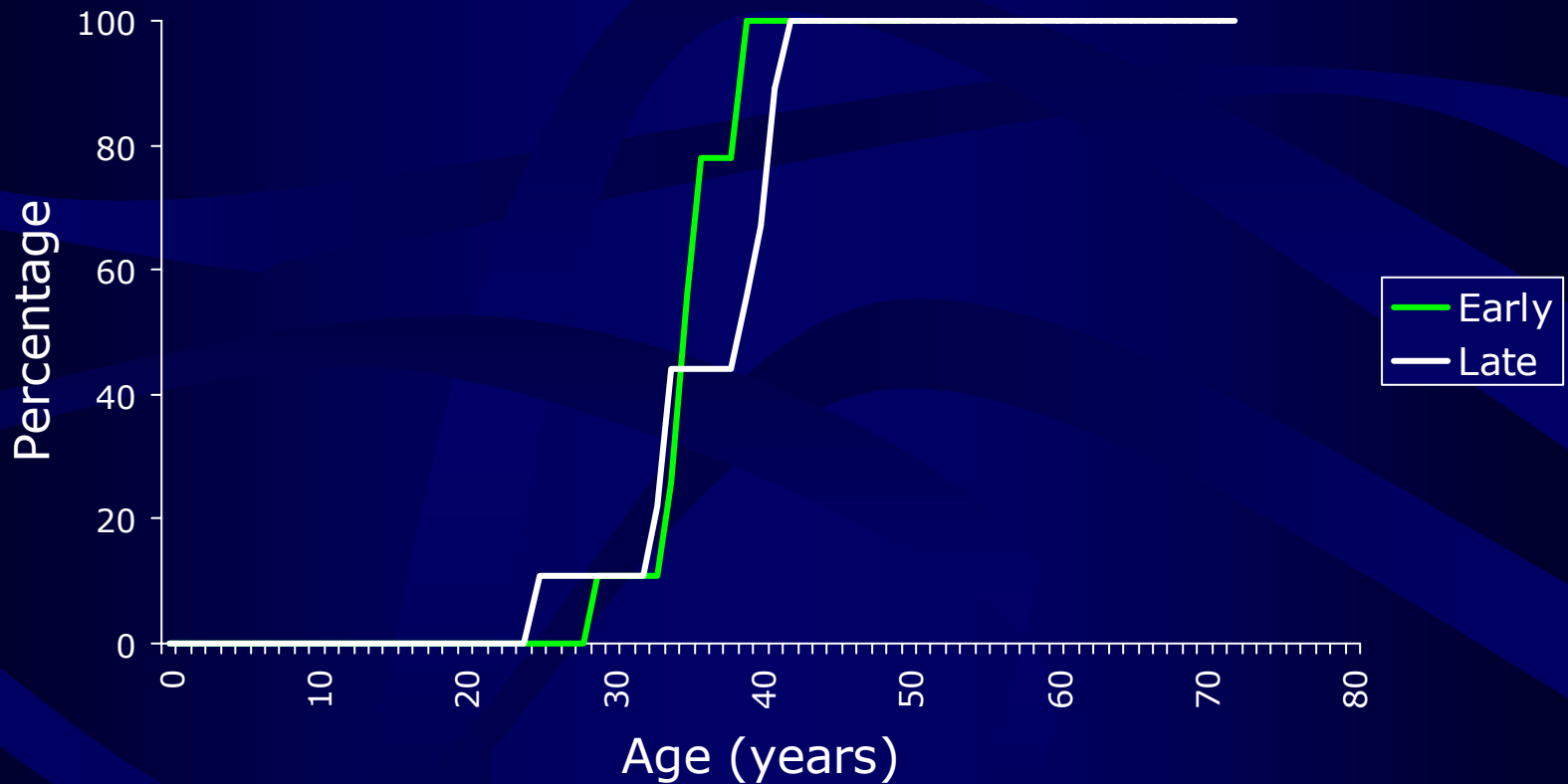
Difference between start of joint and spine problems



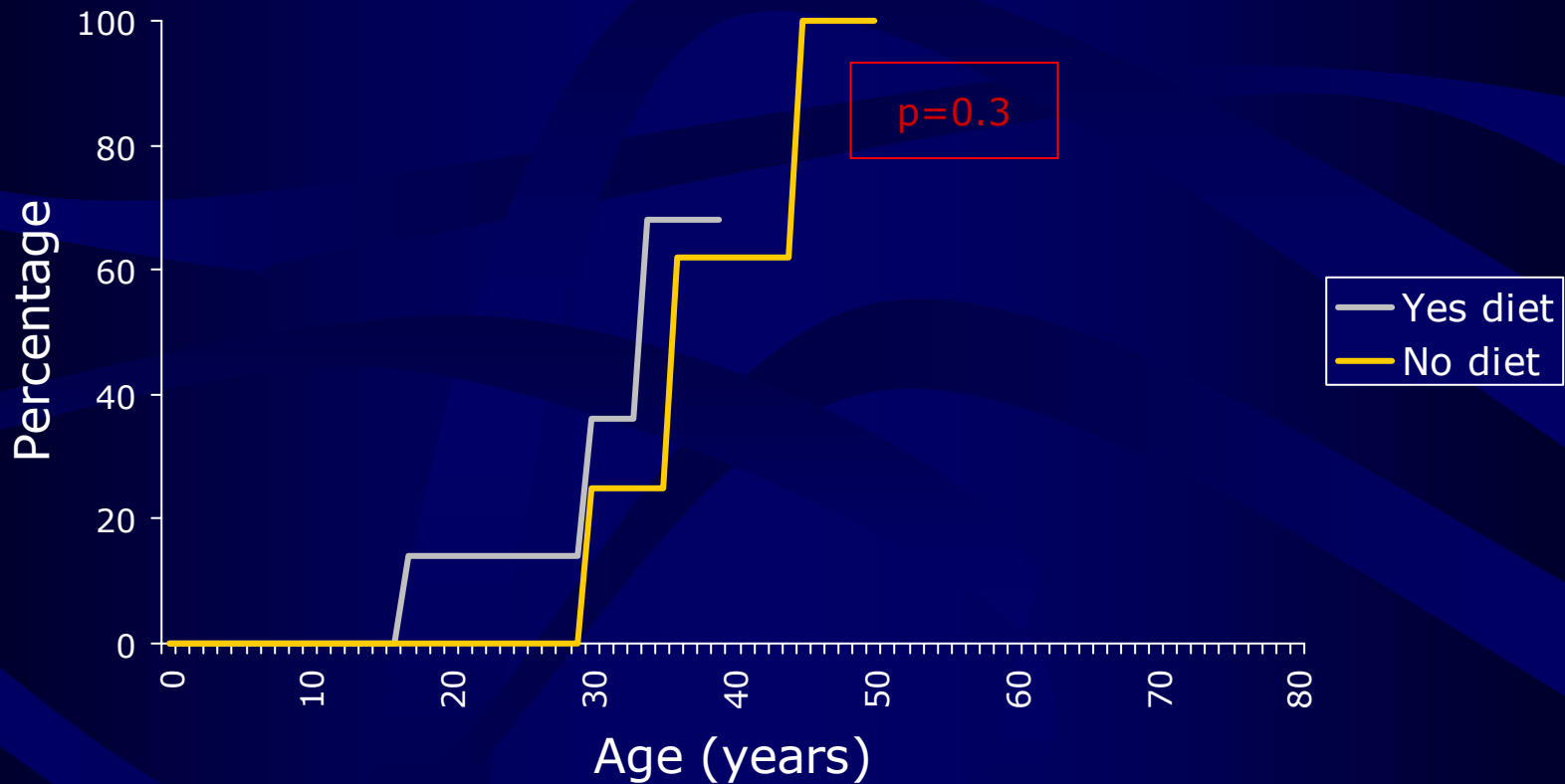
First symptom joints according to time of diagnosis



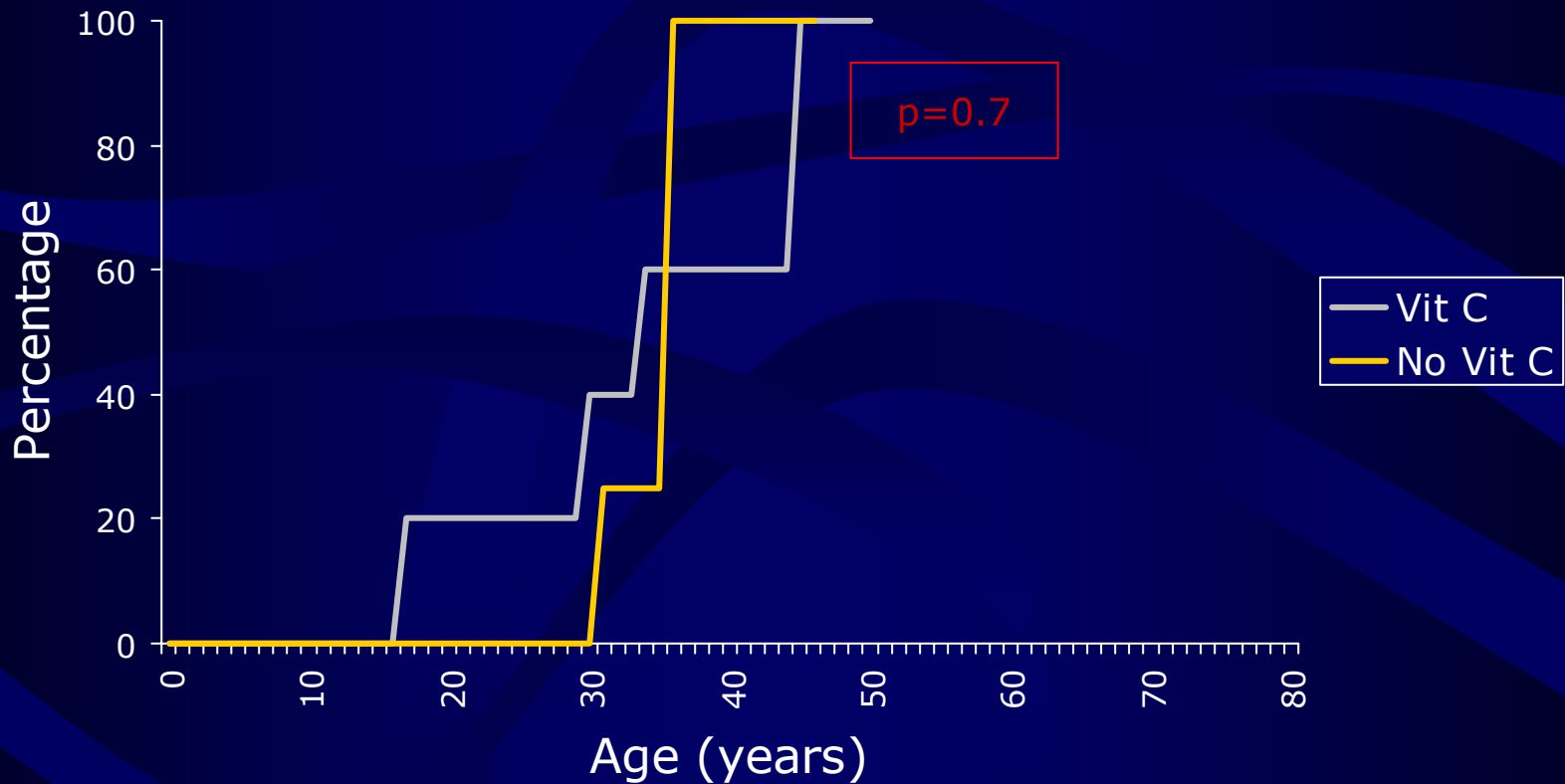
First symptom spine according to time of diagnosis



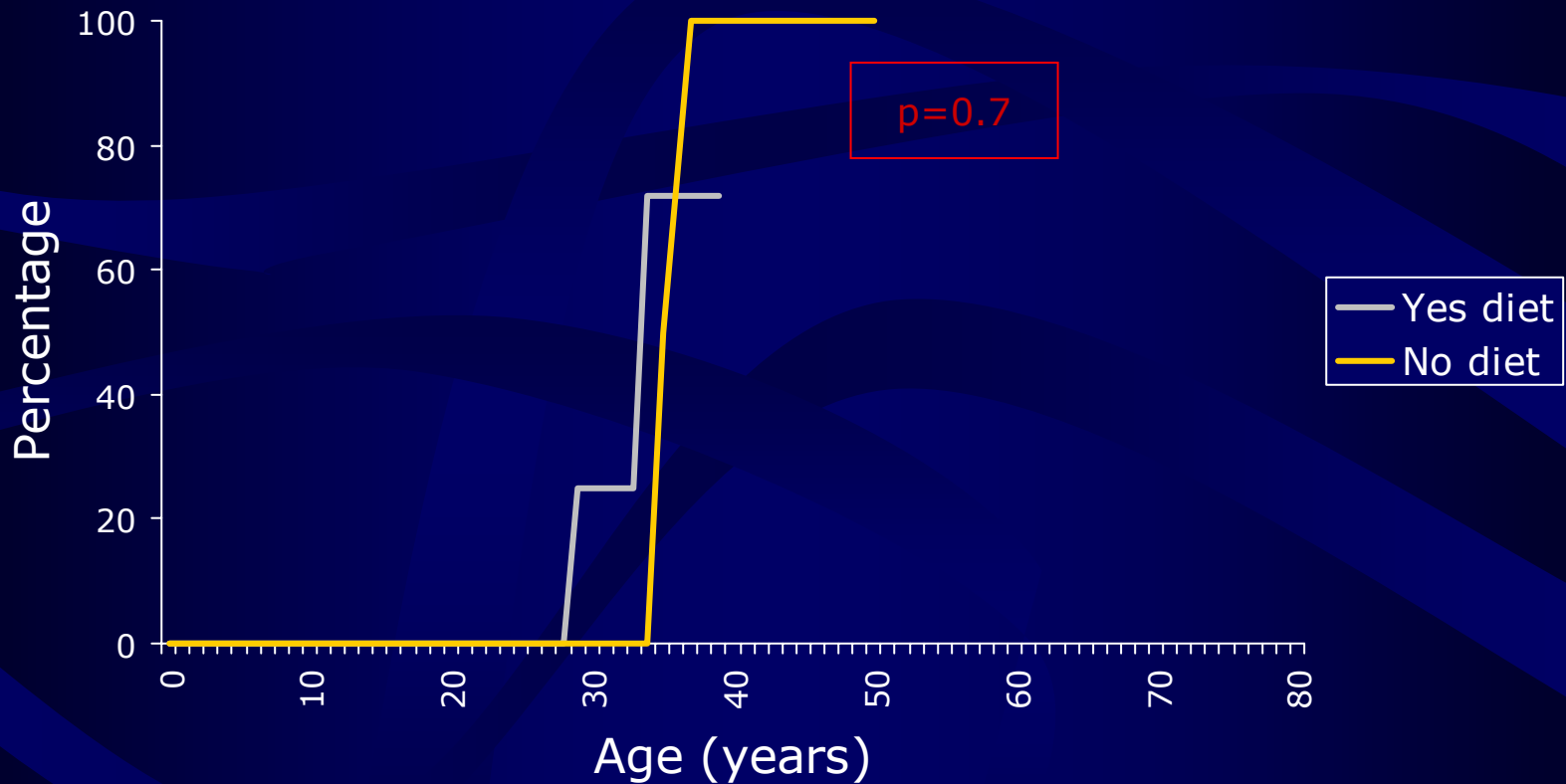
First symptom joints with early diagnosis according to diet



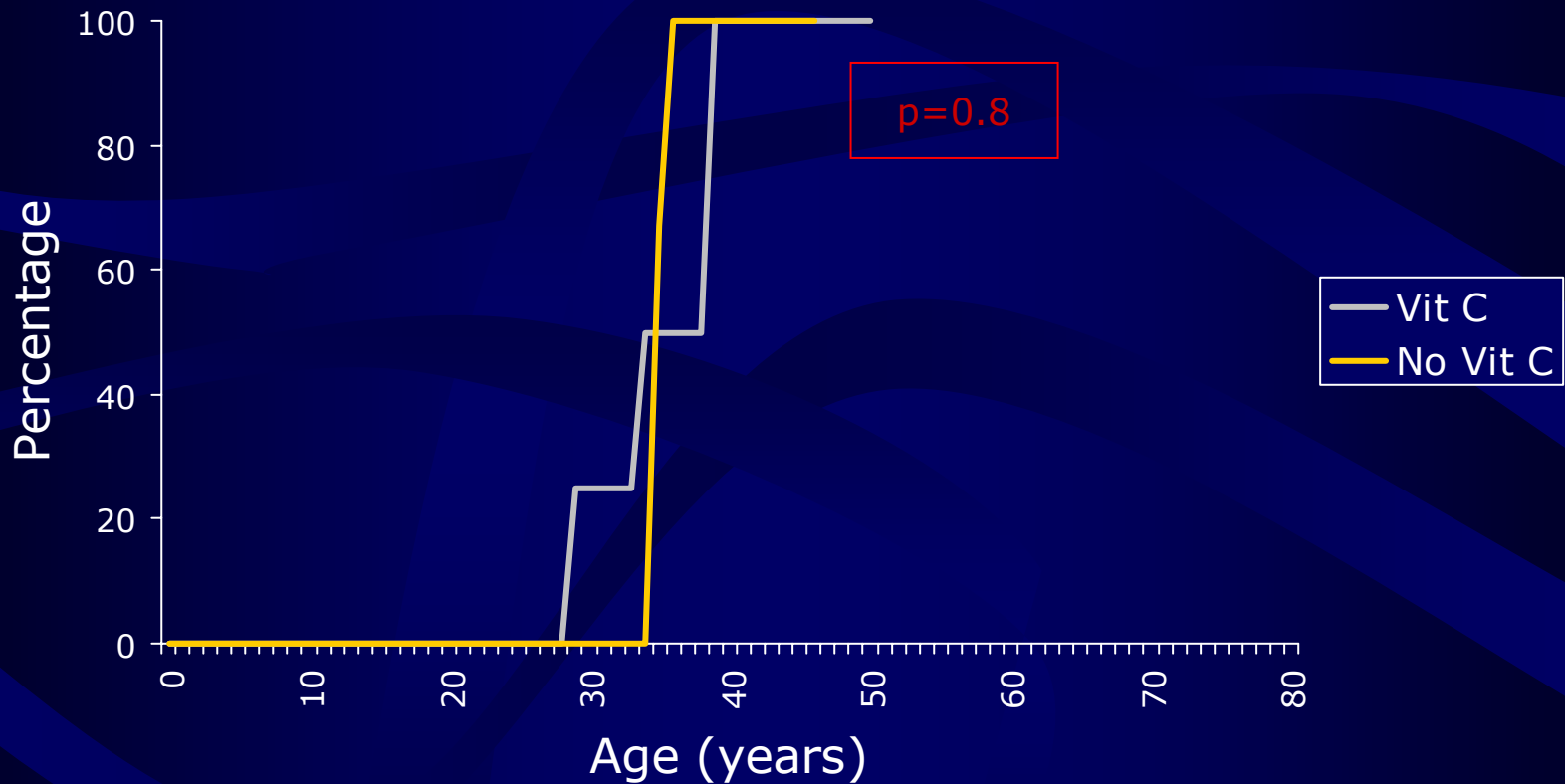
First symptom joints with early diagnosis according to vitamin C



First symptom spine with early diagnosis according to diet



First symptom spine with early diagnosis according to vitamin C



The Dutch survey

Profession:

- Construction 6
- Student/teacher 3
- Administration 3
- Logistical support 3
- Medical/paramedical 2
- Security 1
- Theatre 1
- Dentist 1
- Retired 3

Similar age-relations with construction workers

The Dutch survey

Ultrasound evaluation:

- Valve replacement in 1 patient
- No abnormalities in all the others

The Dutch survey

New events:

5 joint replacements in 2 years

The Dutch survey

Treatment:

- Protein restriction at any time: 44%
- Vitamin C at any time: 42%

Current treatment

- ❑ Protein restriction
- ❑ High doses of vitamin C

Genetic defect

Site of intervention

PR

Excess HGA

Excess HGA in plasma

Excess HGA in urine

Polyphenol oxidase in tissue

Polymers

Binding to sulhydrylgroups

BQA

Binding to collagen

Vit C

Free radical damage

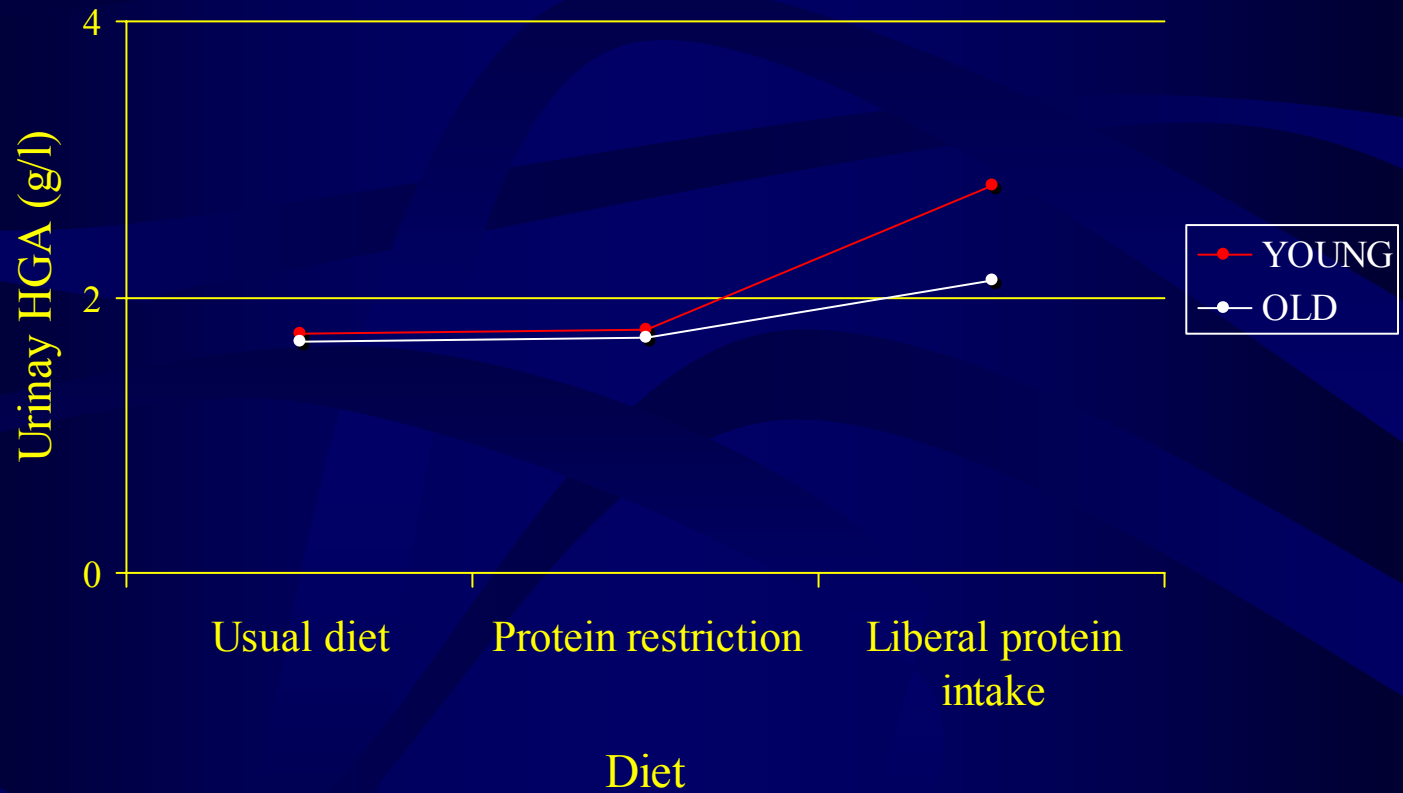
Cartilage damage

Joint disease

Current treatment

- ❑ Protein restriction
 - Difficult to maintain at higher ages and less effective

Dietary manipulations in alkaptonuria



Current treatment

- ❑ Protein restriction
 - Difficult to maintain at higher ages and less effective
 - ❑ High doses of vitamin C
 - High doses may even be pro-oxidative
- What is the evidence
- Does it justify the burden of treatment?

Case report

- A male (FZ) born 28-8-1963
- Referred by paediatrician from the AMC Amsterdam
- First diagnosis made within the first year: discoloration of urine
- Period of treatment with vitamin C and protein restriction until 12th year
- Surgery left knee (meniscus)
- Second diagnosis made in 1998 (35 year) with the first operation because of a lumbar hernia nuclei pulposi

Case report

- No other complications related to alkaptonuria
- Physical examination: dark cerumen, darkened helices
- Limited mobility in all directions of the shoulder joints
- Increased kyphosis of the spine
- No other abnormalities
- Radiographic examination:
 - Cervical spine: little exophytes
 - Thoracic spine: no abnormalities
 - Lumbo-sacral spine: discopathy all levels with subchondral sclerosis
 - SI-joints normal
 - Hips: no abnormalities
 - Knee: some narrowing of the joint space
- Ultrasound: no evidence of valve abnormalities

Case report

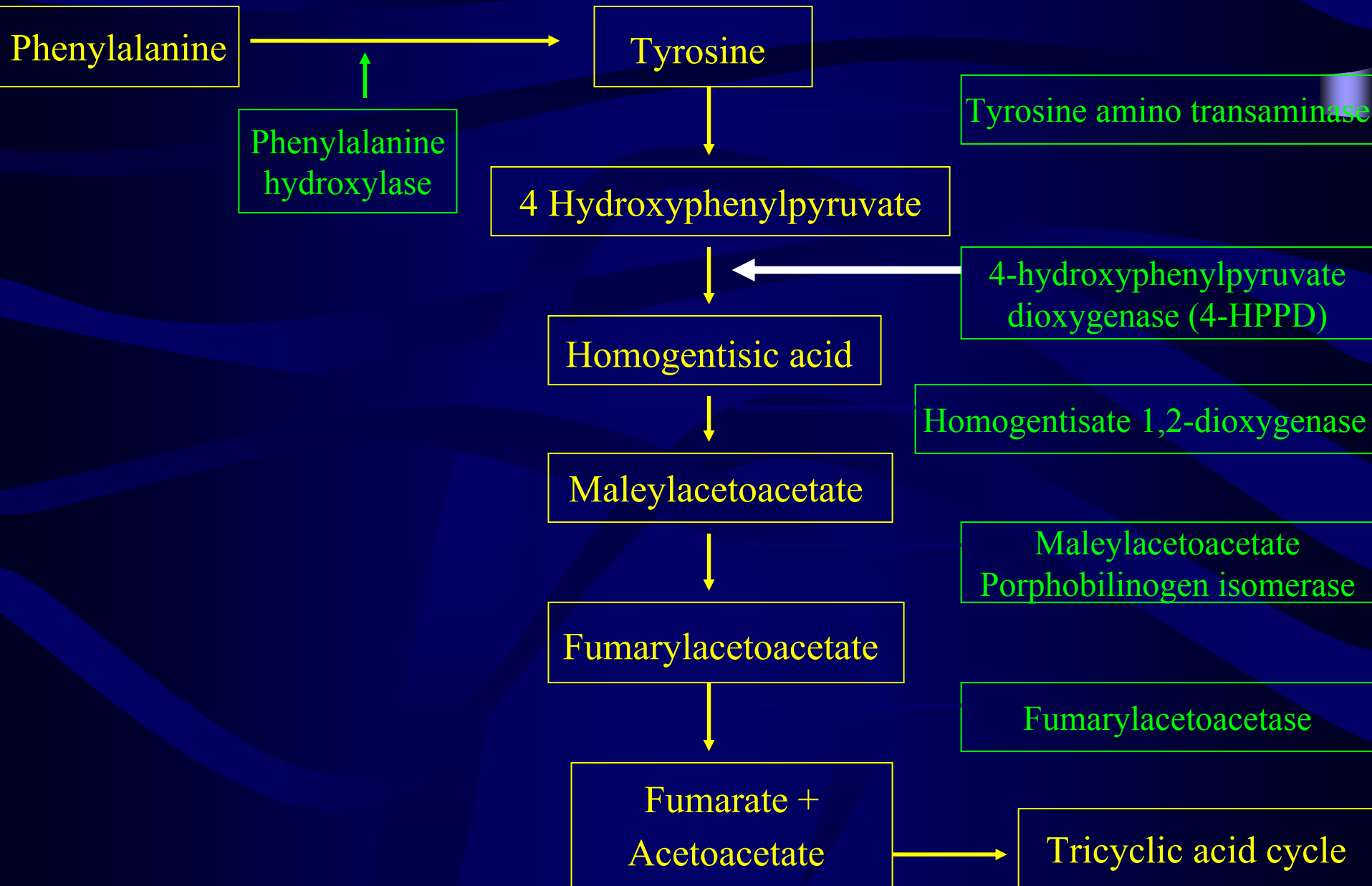
- Urine: large excretion of homogentisic acid
- Referral to the dept. of Neurology: radicular syndrome, no substrate?
- MRI: multiple sequesters
- 2001: Surgery
- No great improvement in symptoms.
- His questions:
 - What does the future hold?
 - What is the best treatment?
 - Which professions would be advisable?

New treatment

NTBC (Nitisinone)

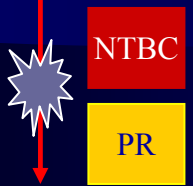
- Initially tested as a herbicide
- Inhibits competitively an upstream enzyme:
4-hydroxyphenylpyruvate dioxygenase (4-HPPD)
- Inhibits the overproduction of homogentisic acid
- Lower plasma homogentisic acid levels and no urinary homogentisic acid production
- Recently obtained the orphan drug designation from EMEA (European Medicine Evaluation Agency)

Site of action of NTBC



Genetic defect

Site of intervention



Excess HGA

Excess HGA in plasma

Excess HGA in urine

Polyphenol oxidase in tissue

Polymers

Binding to sulhydrylgroups

BQA

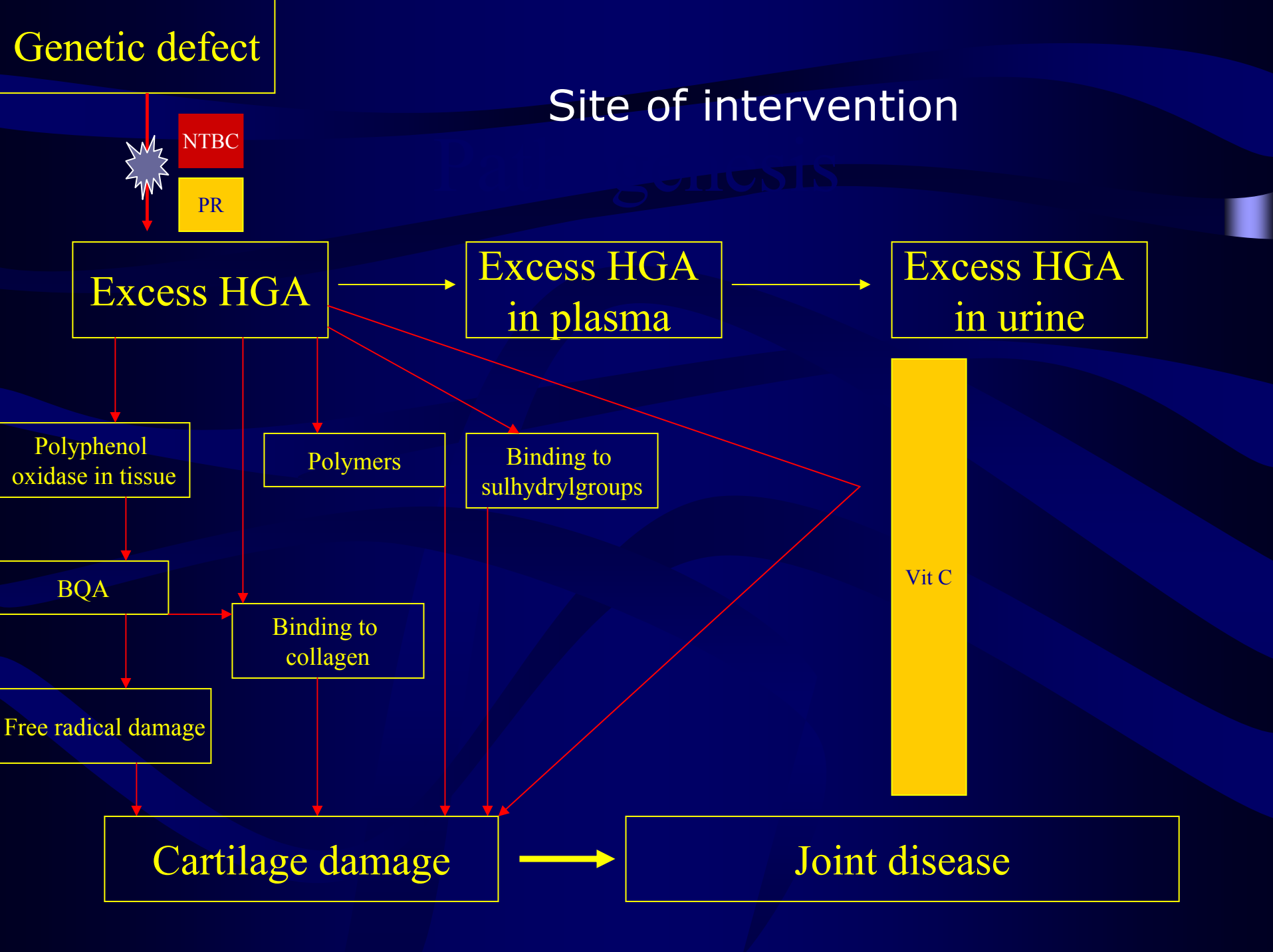
Binding to collagen

Vit C

Free radical damage

Cartilage damage

Joint disease



(Orphan) drug development

□ Phase 1:

- Testing in the laboratory to get some estimation of the drug doses required
- Toxicity: reproductive system
- Testing in healthy volunteers whether it is not directly lethal

□ Phase 2:

- A: Dose finding study in patients
- B: Longer term exposure in patients

□ Phase 3:

- Testing against usual care in a randomized trial

□ Phase 4:

- Treatment in larger patients groups and/or for longer periods (post-marketing surveillance)

(Orphan) drug development

Aim of the procedures:

- Development of a pharmacological agent
- Known effectiveness
- Known risks:
 - adverse events (specific, non-specific)
 - reproductive toxicity (female, male)
 - teratogenic risk
- Costs of use (final price at the counter)

NTBC development (1)

□ Phase 1:

- Testing in the laboratory to get some estimation of the drug doses required

Data from the tyrosinaemia experience? 1 mg/kg? Less?

- Toxicity

Reproductive system?

Ocular abnormalities: partially depending on the degree of elevation of the plasma tyrosine level (>500-600 $\mu\text{mol/l}$).

Reversible. Animals: in rats and beagle, but not in mice or rabbits.

NTBC development (2)

□ Phase 1:

- Safety

Not lethal in man

- Pharmacology:

Non-competitive inhibitor

T_{1/2} nitisinone: 54 hours

T_{1/2} tyrosine: 7 days!

NTBC development (3)

□ Phase 2:

- Proper dose finding in patients:
2003
- Extension study with multiple dosing
2003

□ Phase 3:

- Proper randomized, placebo-controlled clinical trial
2004

□ Phase 4

Compassionate use: with consent of the patient in cases of a mortal disease

A clinical research effort in alkaptonuria

Nice idea, but.....

- Alkaptonuria is a rare disease
- Complications in alkaptonuria develop slowly
- Do we use NTBC and/or other strategies?
- Where does the funding come from?
- How about collaboration with industry?

Hypercholesterolaemia?

Erectile dysfunction?

Fabry?

After statins

After viagra

After α - or β -galactosidase

Partir (from Paris) c'est
mourir un peu, mais.....

ne pas rêver c'est être
complètement mort

Short outline of the clinical research programme

- Description of the currently known patients (survey), initially in the Netherlands with later a European extension
- Follow-up to describe prognosis and event rate: ***outcome analysis of unselected patients***
- Pre-clinical testing of NTBC
- Clinical testing of NTBC

Why is outcome analysis in
unselected patient groups
so extremely important?

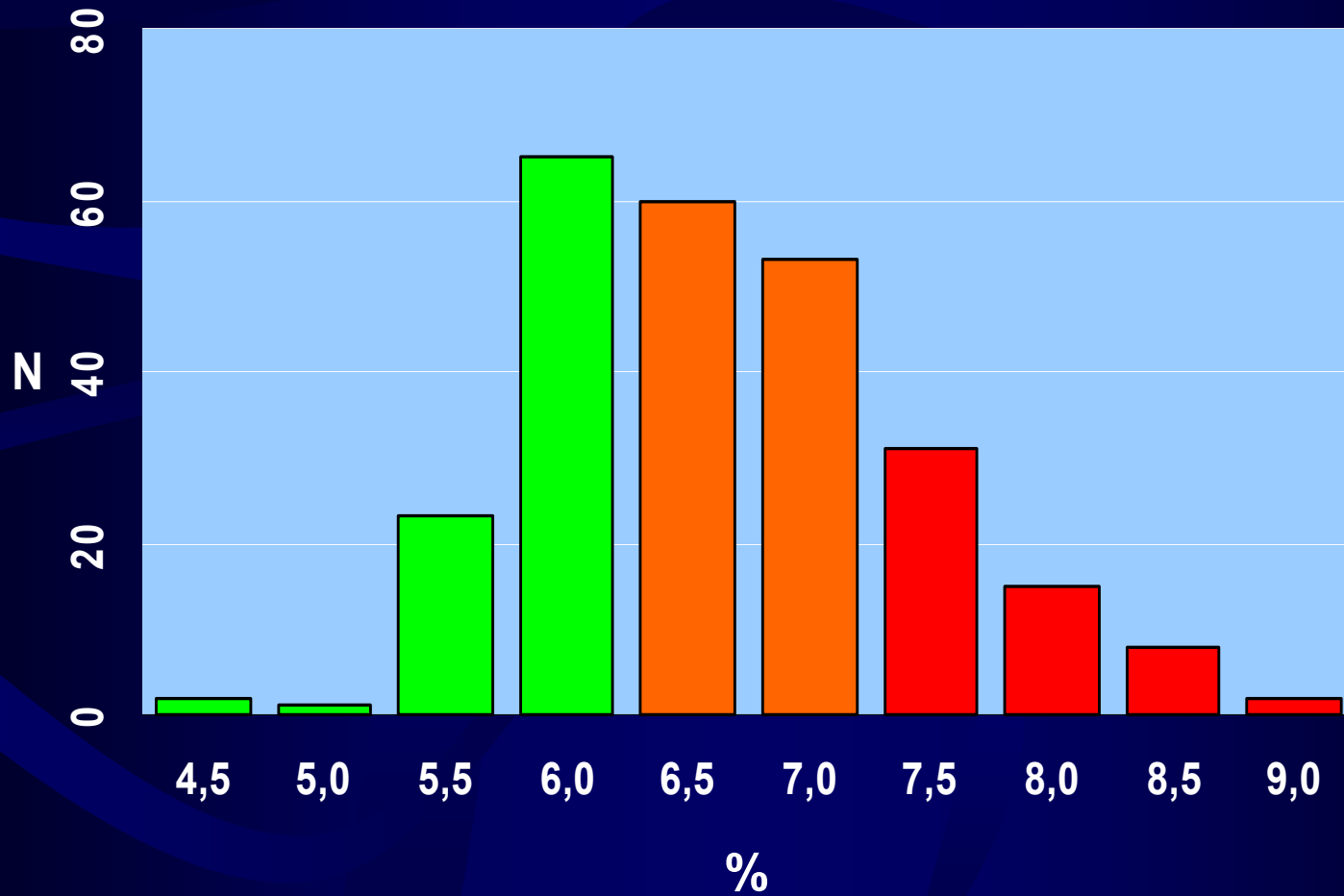
Design

- Inclusion of all Dutch women with type 1 diabetes mellitus becoming pregnant in a period of 12 months
- Participation of all Dutch hospitals (118)
 - Internist
 - Gynaecologist
 - Diabetes-nurse
 - (Patients themselves)
- Completing questionnaires (internist, gynaecologist, paediatrician, and patient)
- Study duration of 4 years

Preconceptional care and knowledge

- 84% of pregnancies are planned
- 70% start with folic acid before conception
- HbA1c at 10 weeks pregnancy: 72% \leq 7.0%
- Problem population: lower education, foreign extraction

HbA1c at 10 weeks



Frequency and risk factors for severe hypoglycaemia (SH) in the first trimester

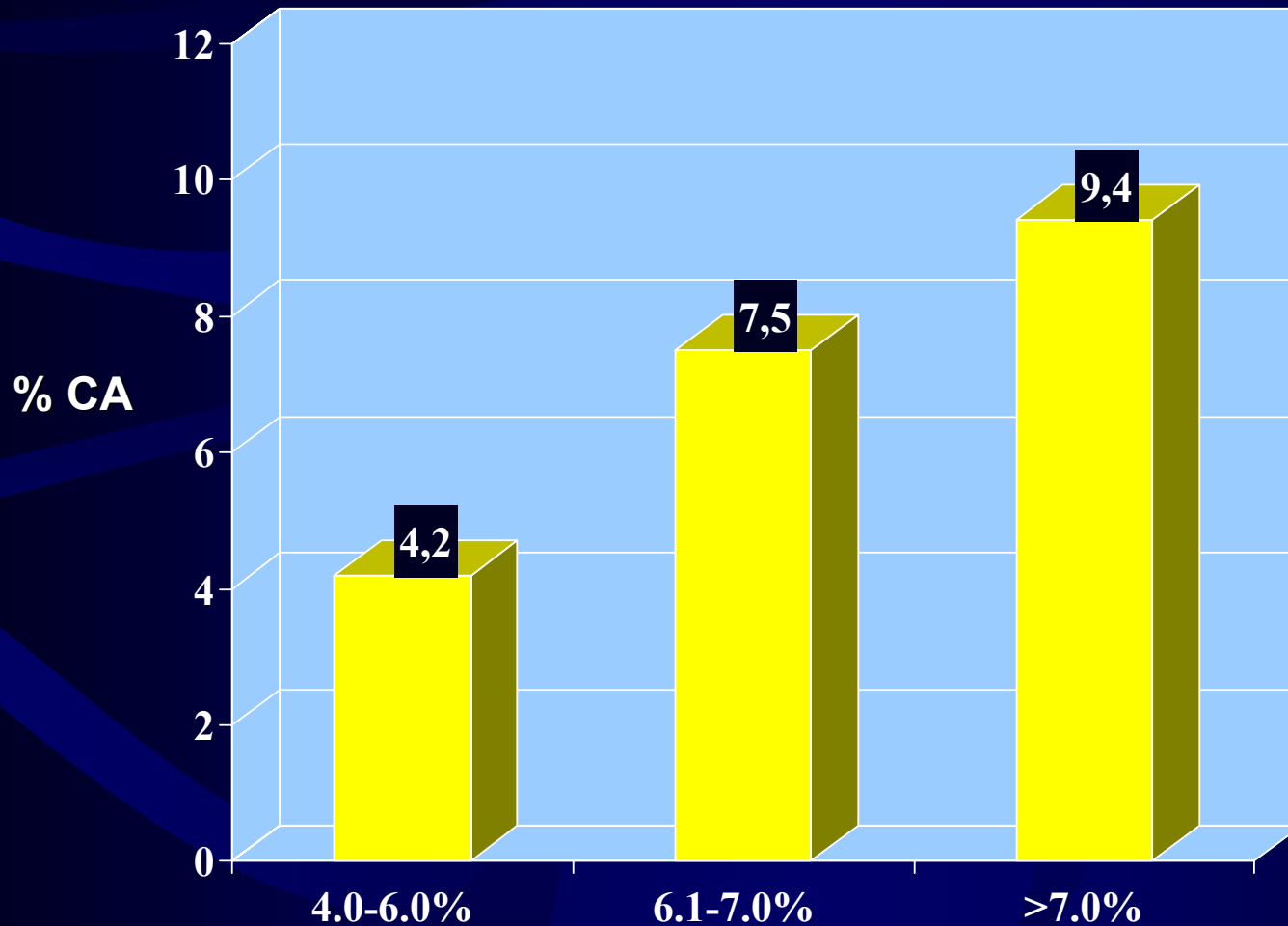
	Preconceptional	1e trimester
Frequency SH (episodes/4 mts)		
SH (all episodes)	0.9 ± 2.4	2.6 ± 6.3 *
Hypoglycaemic coma	0.3 ± 1.3	0.7 ± 3.7 *
Proportion of women:		
SH (all)	25%	41% *
Uncomplicated SH	16%	22% *
Coma	9%	19% *

* p<0.001
N=278

Complications (1) *congenital abnormalities*

- Major 4.9%
- Minor 3.4%
- Total 8.3% (95% CI 5.3-11.3%; 2%, $P < 0.05$)
- Unplanned vs. planned pregnancies: major 10.4% vs. 3.8% ($P = 0.05$)

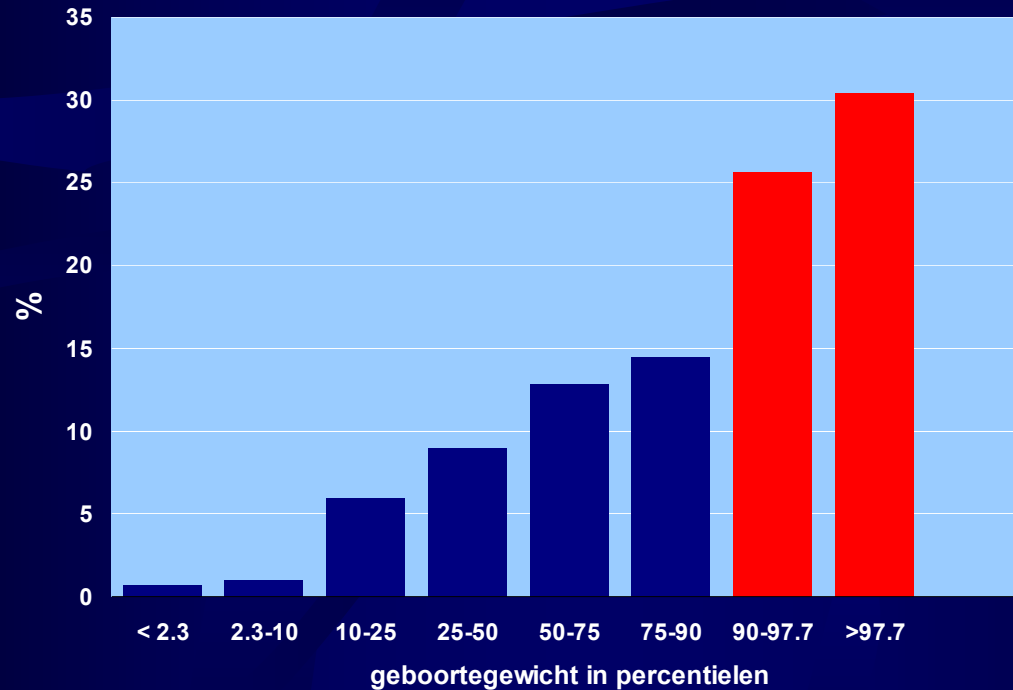
HbA1c at 10 weeks and congenital abnormalities



Complications (2)

- Perinatal mortality: 2.8% (95% CI 1.0-4.6%; 0.9%, $P < 0.05$)
 - CM (n=4), IUVD (n=2), PE (n=2), asphyxia (n=1)
 - 2 late neonatal mortality
- Maternal mortality: n=2 (0.6%; 0.01%, $P < 0.05$)
 - Severe hypoglycaemia at 17 weken
 - Amnion fluid embolism durante partu
- Macrosomia ($>p90$) 56.1%
 (**$>p97.7$**) **30.4%**

Birth weight children of mothers with type 1 diabetes



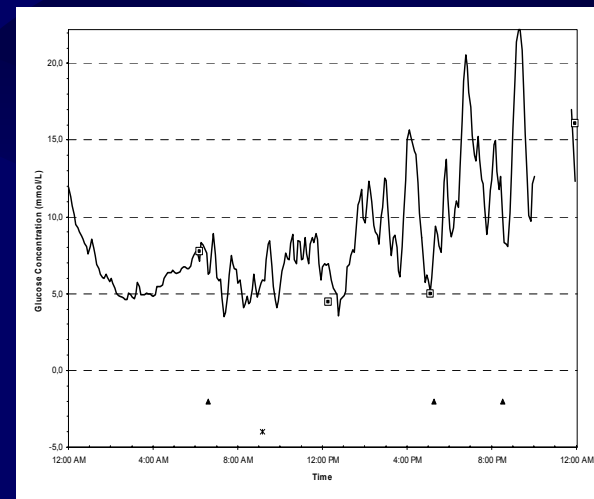
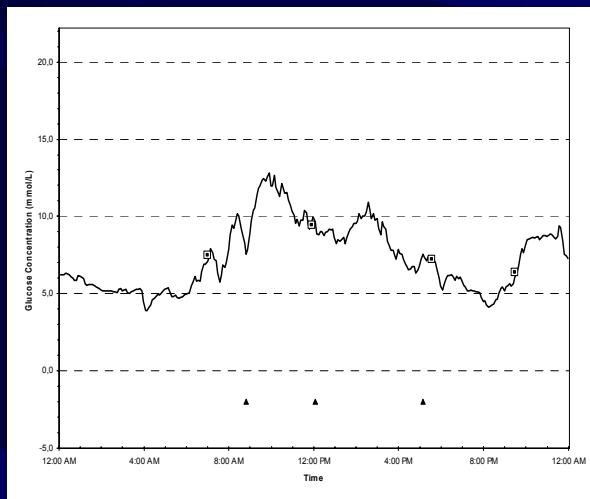
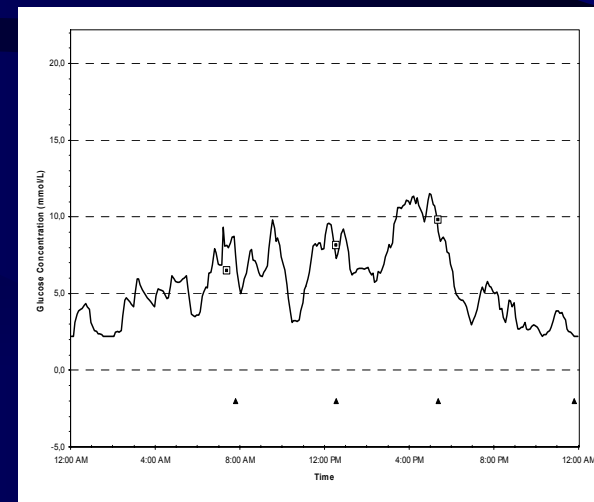
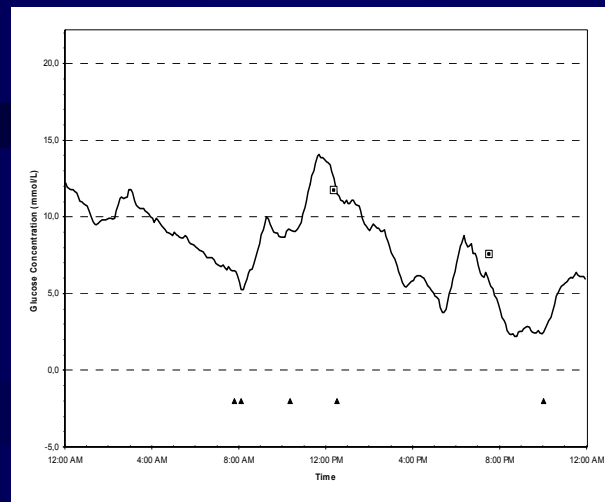
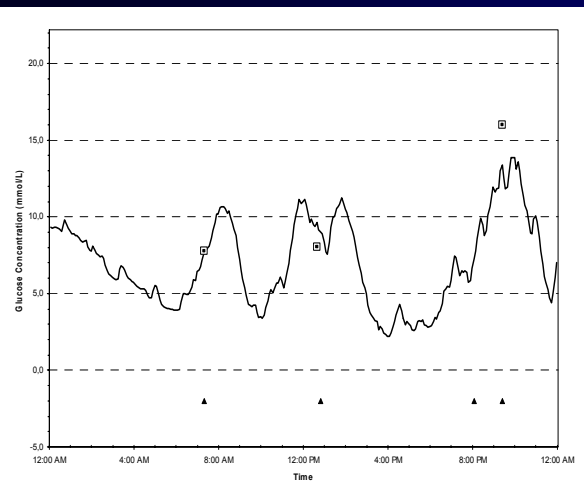
Conclusions

1. Periconceptual preparation often good
2. HbA1c in the majority of women in the 'safe' area
3. Still increased frequency of congenital abnormalities, also within the 'safe' area (HbA1c 6-7%)
4. Very high frequency of macrosomia
5. High frequency of neonatal morbidity
6. High frequency of maternal severe hypoglycaemia
7. Strongly increased maternal mortality

So what now?

1. Is an HbA1c between 6-7% really safe?
2. Is HbA1c a good index of the complexity of glycaemic control: Glucose-variability
3. Should 'defensive' forces be strengthened (anti-oxidantia?)
4. Should we give different information?
5. Type 1 diabetes and pregnancy is still not a won race.
6. The goals of the St. Vincent declaration are not met, may be this is simple not, and who knows never possible.

'Safe' HbA_{1c}: 6.0-7.0% (n=5)



Outcome analysis in unselected patient groups is so extremely important because it

- it provides adequate information on the results of the current therapy
- it can provide new insights leading to new ideas
- it can lead to the development of or use of new pharmacological or non-pharmacological treatments
- it can discard myths
- it can give valuable information of the life situation of patients
- It can identify patients groups at highest risk

Conclusions

- ❑ **Alkaptonuria may be more frequent than 1/500.000**
- ❑ **No current effective treatment**
- ❑ **Impact on life after the end of the third decade**
- ❑ **Further clinical epidemiologic studies are required with outcome analysis and assessment of event rate**
- ❑ **European collaboration is wanted**
- ❑ **NTBC offers a new and exciting pharmacological treatment possibility**
- ❑ **But it remains a 'normal' drug with possible serious side effects, used in a non-life-threatening disease**
- ❑ **European collaboration is wanted**

LE FIN

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