Prednisolone-treatment induces hepatic insulin resistance in C57Bl/6J mice, measured by a newly developed whole body glucose test

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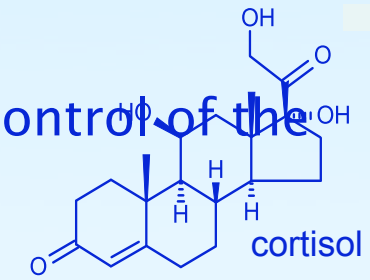
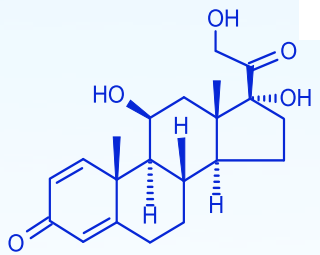
Introduction

Glucocorticoids



cortisol

- Hormones produced by the adrenal gland under control of the hypothalamic-pituiary-adrenal axis
- Cortisol, corticosterone in rodents
- Endogenous role in salt and water metabolism, blood pressure, glucose metabolism, immune system
- Synthetic glucocorticoids used as immunosuppressive drugs in chronic inflammatory diseases



prednisolone

Severe side effects

Introduction

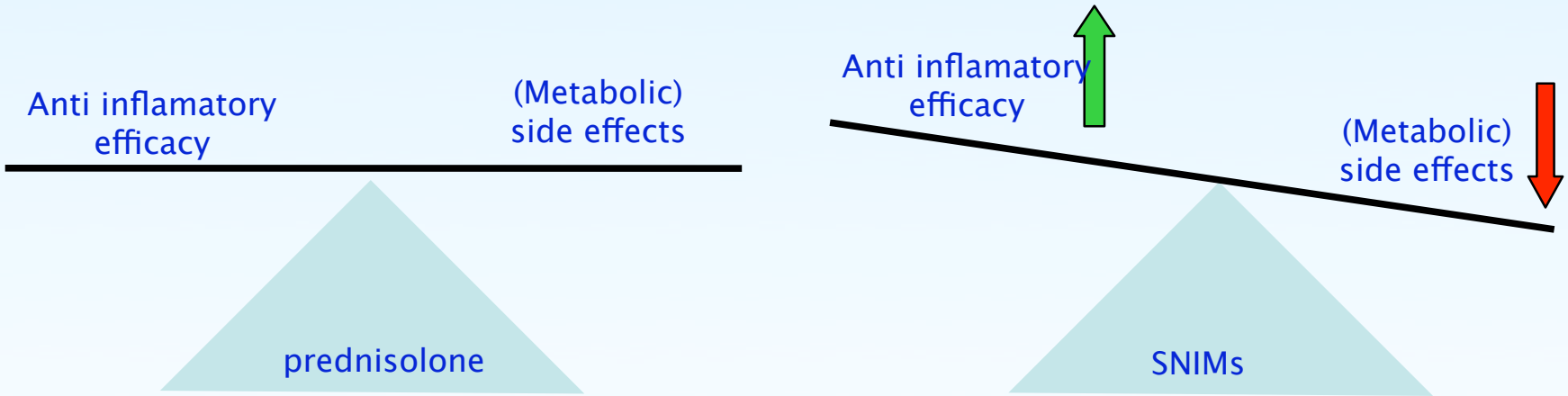
- Cardiovascular diseases
- Mood swings/ depression
- Weight gain
- Fat redistribution
- Hypertension
- Insulin resistance
- Growth retardation
- Impaired immune respons
- Skin thinning
- Muscle wasting
- Osteoperosis
- Moon face
- Insomnia

Cushing syndrome

TiPharma Project

Project T1-106

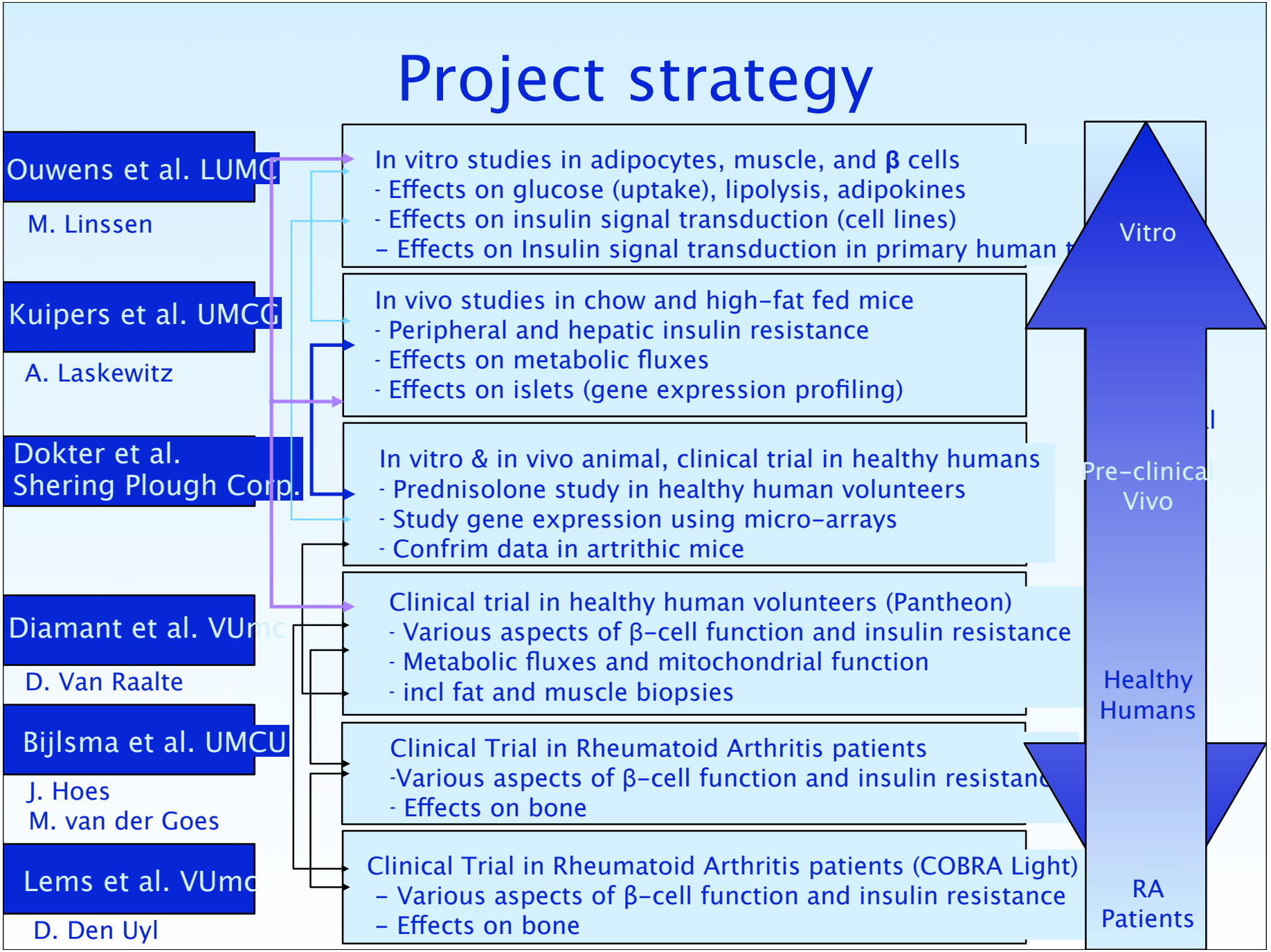
Glucocorticoid-induced insulin resistance



Aim

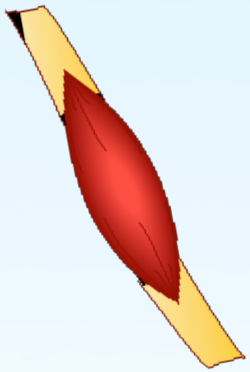
Identify the most important tissue(s) and mechanism(s)
by which prednisolone induces metabolic side effects

Project strategy



Organs most likely to be involved

Muscle
Adipose

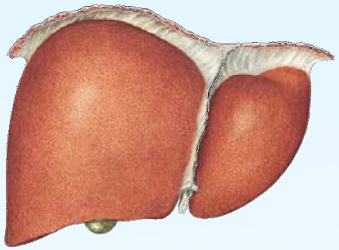


- insulin resistance
- glucose uptake
- muscle wasting

Brain

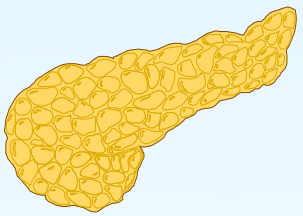
- insulin resistance
- glucose uptake
- mineralocorticoid action
- behavioural changes

Liver

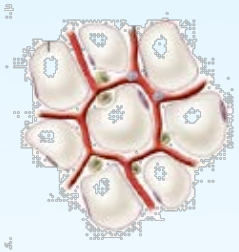


- insulin resistance
- glucose production
- glucose uptake

Pancreas



- insulin resistance
- insulin secretion
- glucose sensing



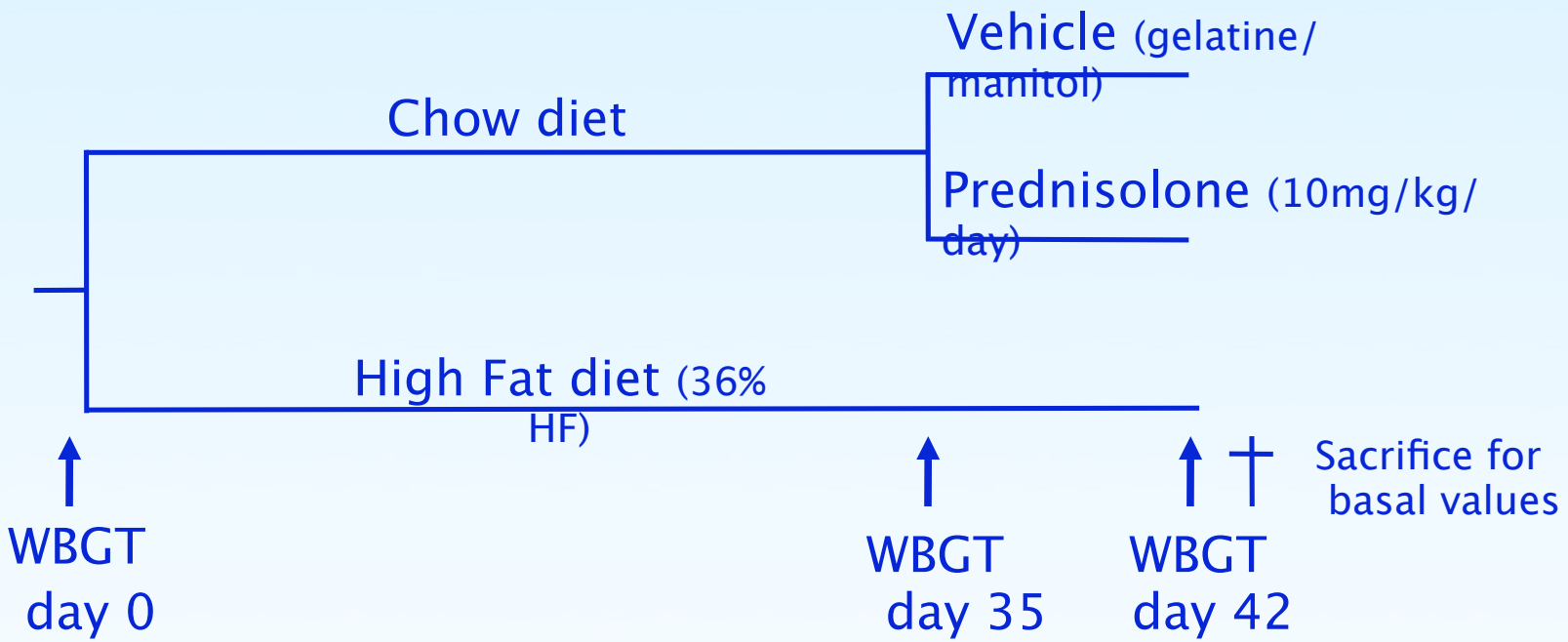
- insulin resistance
- glucose uptake
- changes body fat content (chronic)
- change in adipokines

Aim

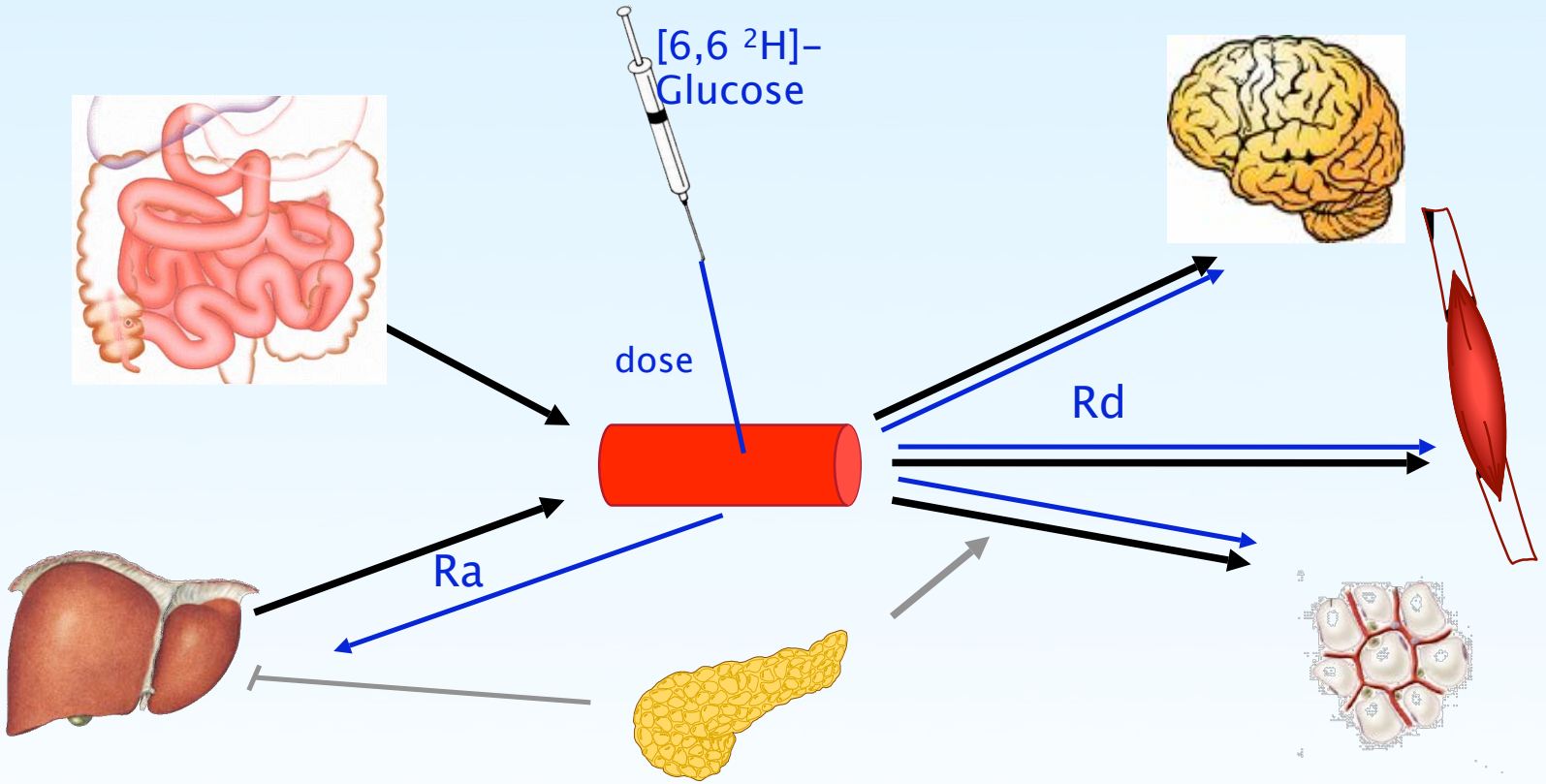
Determine the roles of liver and peripheral tissues in glucocorticoid-induced insulin resistance, employing mouse models

Measure development of insulin resistance by whole body glucose test

Experimental set-up

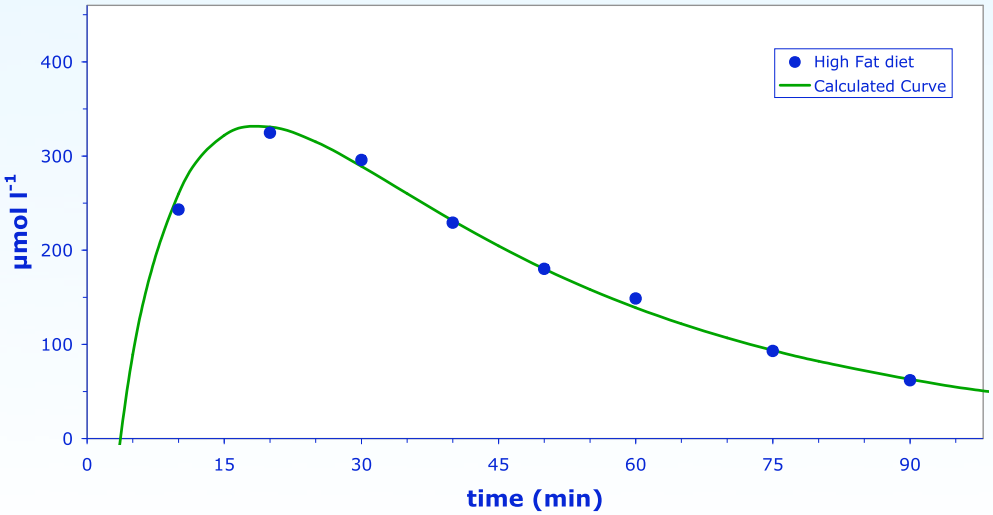


Method Whole Body Glucose Test



Method Whole Body Glucose Test

- Inject ip trace amount [6,6-^{2}H]-glucose
- Measure blood glucose levels every 10 min
- Take a blood spot on filter paper every 10 min
- Extract glucose from bloodspots and derivatise to glucose-pentaacetate
- Analyse by GC-MS to obtain fractional contribution of [6,6-^{2}H]-glucose



$C_t = (C^{el}_0 * e^{-kelt}) - (C^{ab}_0 * e^{-kabt})$

Method Whole Body Glucose Test

Parameter		Formula
[6,6-^{2}H]-glucose blood concentration	C_t	$= C_0 * e^{-kt}$
	C^{el}_t	$= C^{el}_0 * e^{-kelt}$
	C^{ab}_t	$= C^{ab}_0 * e^{-kabt}. C^{el}_0$
[6,6-^{2}H]-glucose in blood over time	C_t	$= (C^{el}_0 * e^{-kelt}) - (C^{ab}_0 * e^{-kabt})$
Lag time	t_{lag}	$= (\ln(C^{ab}_0) - \ln(C^{el}_0)) / (k_{ab} - k_{el})$
[6,6-^{2}H]-glucose blood concentration at t_{lag}	C_{lag}	$= C^{el}_0 * e^{-keltlag}$
	C_{lag}	$= C^{ab}_0 * e^{-kabtlag}$
Bioavailability	F	$= 1 - ((C^{ab-}_0 * k_{el}) / (k_{ab} * C^{el}_0))$
Volume of distribution	V	$= (F * D) / C_{lag}$
Amount of glucose in the sampled pool	A	$= V * [glc]$

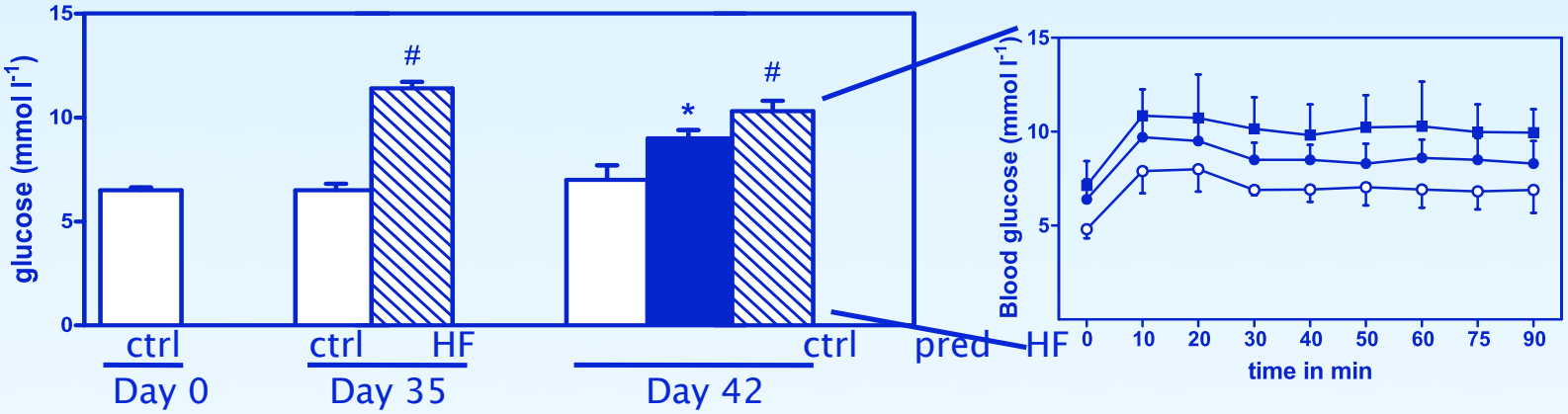
Results

Basal values

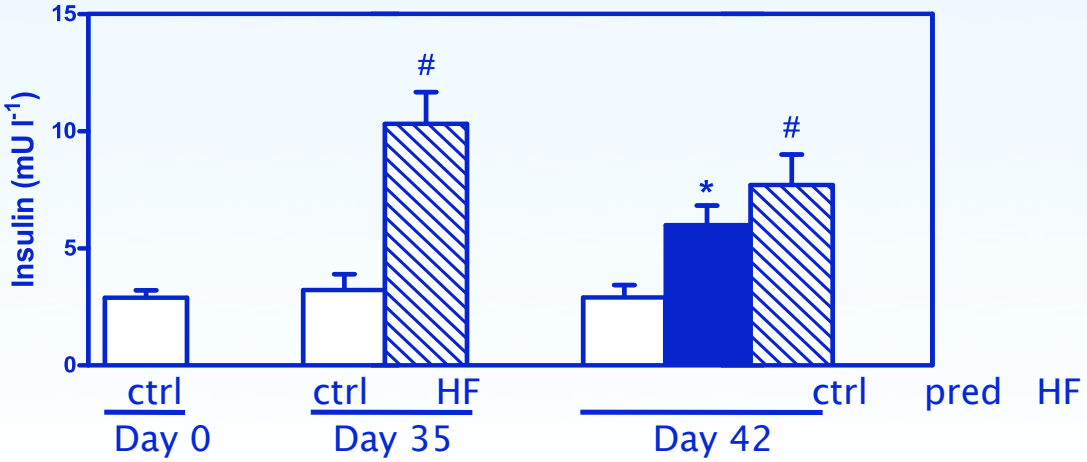
Plasma	Control	Prednisolone	High fat
Glucose fasted (mmol/l)	7.37 ± 1.45	8.79 ± 2.00	7.58 ± 0.89
Insulin (mU/l)	1.44 ± 0.15	**2.9 ± 1.34 ***	**7.40 ± 6.80 #**
HOMA	0.96 ± 0.47	2.32 ± 2.59	**3.59 ± 3.44 #**
Corticosterone (ug/l)	889 ± 196	**167 ± 116 ***	820 ± 98
Cholesterol (mM)	2.96 ± 0.32	3.38 ± 0.61	**5.85 ± 0.41 #**
Triglycerides (mM)	0.74 ± 0.19	**1.03 ± 0.28 ***	**1.29 ± 0.18 #**
NEFA (μM)	472 ± 76	449 ± 99	580 ± 72
Liver			
Cholesterol (nmol/mg liver)	10.80 ± 0.88	9.59 ± 1.93	11.77 ± 1.99
Free Cholesterol (nmol/mg liver)	7.41 ± 0.88	6.43 ± 1.17	7.19 ± 1.53
Triglycerides (nmol/mg liver)	19.37 ± 8.48	17.18 ± 6.70	**43.72 ± 16.92#**
Glycogen (umol/gr)	91± 72	97 ± 113	111 ± 76

Results

Average glucose levels during whole body glucose test

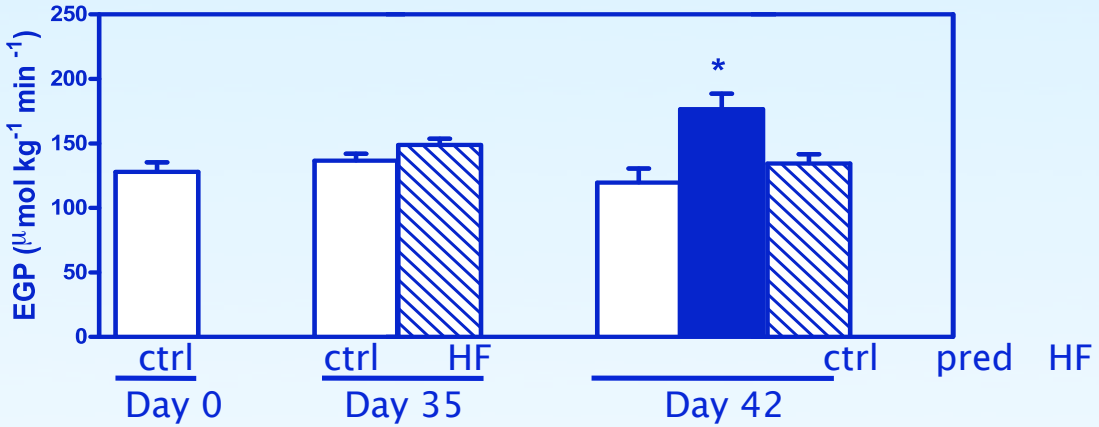


Average insulin levels after whole body glucose test

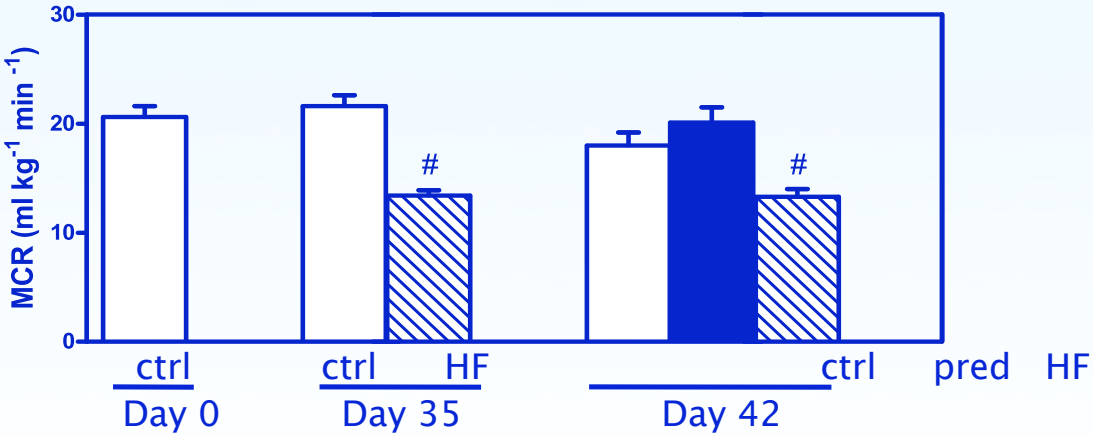


Results

Endogenous glucose production by whole body glucose test



Metabolic clearance rate whole body glucose test



Conclusion

- Prednisolone treatment induces hepatic insulin resistance, shown by
  increased endogenous glucose production

- A high fat diet induces peripheral insulin resistance, shown by
  decreased metabolic clearance

- The whole body glucose test is a good method to measure and disscriminate
  between hepatic and peripheral insulin resistance
→Using stable isotopes more information can be obtained from one animal

Conclusion

Whole body glucose test versus 'gold standard' hyperinsulinemic euglycemic clamp

Advantages

1- no surgery
2- fast
3- less invasive
4- repeatable, so you can measure development over time
5- cheaper
6- basal situation, not a tolerance test
7- own control

Dissadvantage

1- less information about insulin levels
2- glucose and insulin levels are not adjustable
3- not hyperinsulinemic

Acknowledgements

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