The relevance of adipokines as biomarkers for metabolic health in dairy cows

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Outline

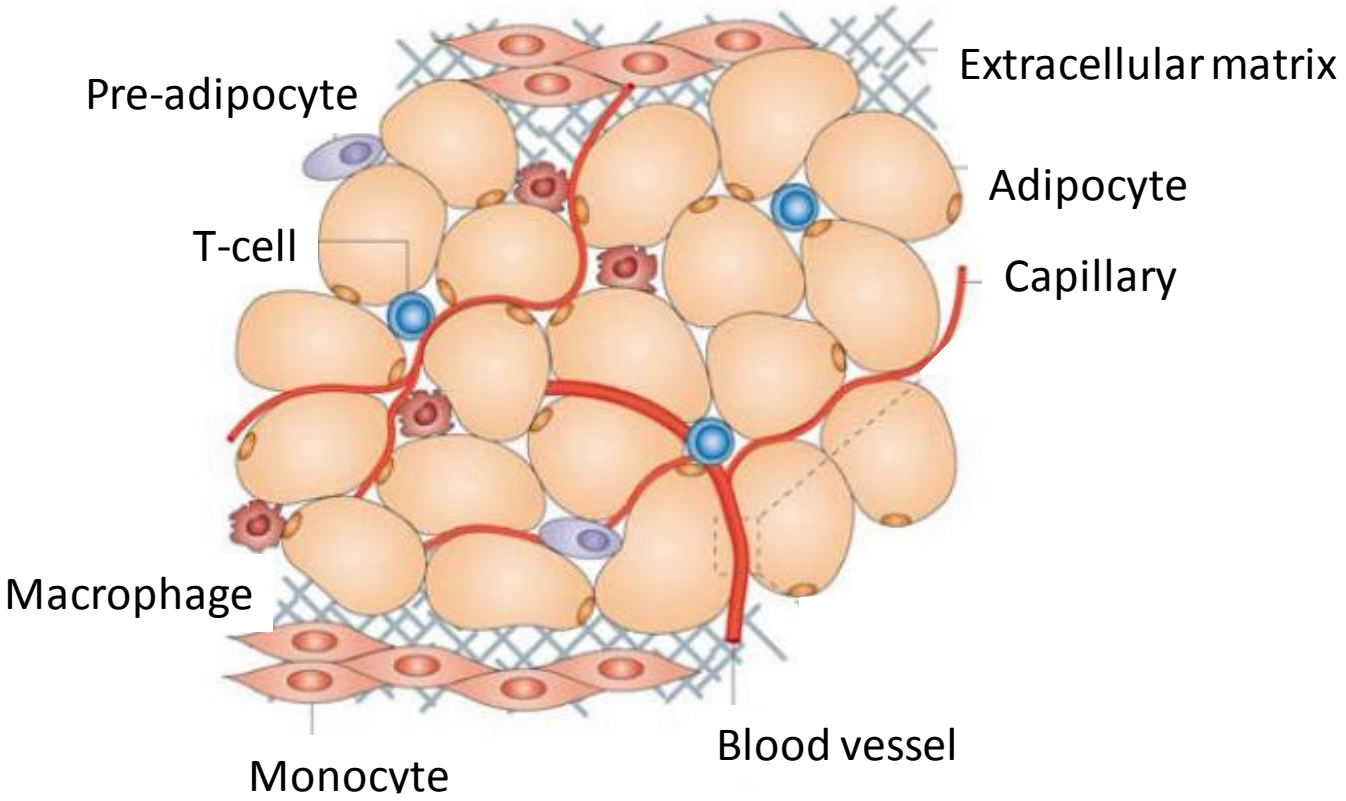
- Adipokines and adipose tissue: definition, general basics, functions
- Metabolic health: Transition cow problems
- Specific adipokines during the transition period: adiponectin, apelin, leptin, resistin
- Critical assessment of the applicability as biomarkers

Adipokines - general background

Adipokines: messenger molecules from adipose tissue

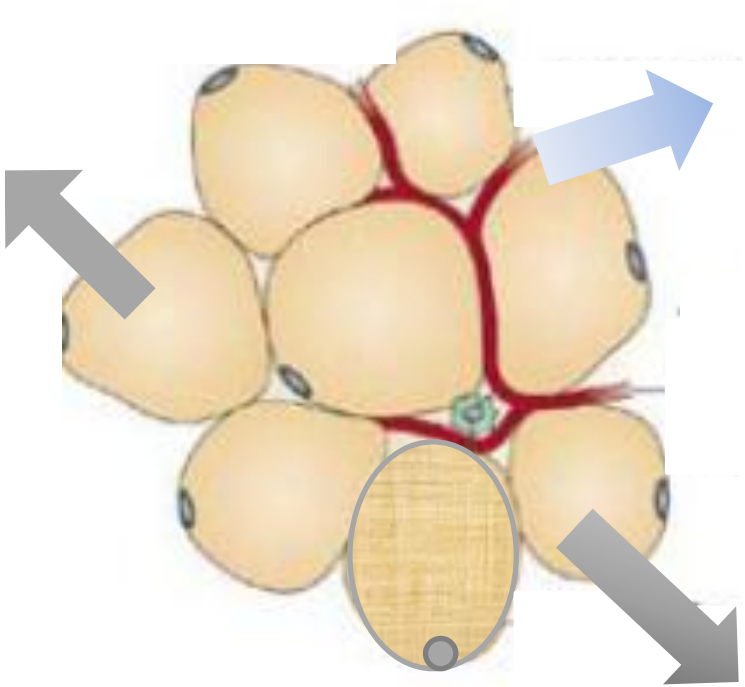
adipose tissue

Stromalvascular cells
(SVC)



Adipokines: the voice of the adipose tissue

Adipokines with metabolic effects

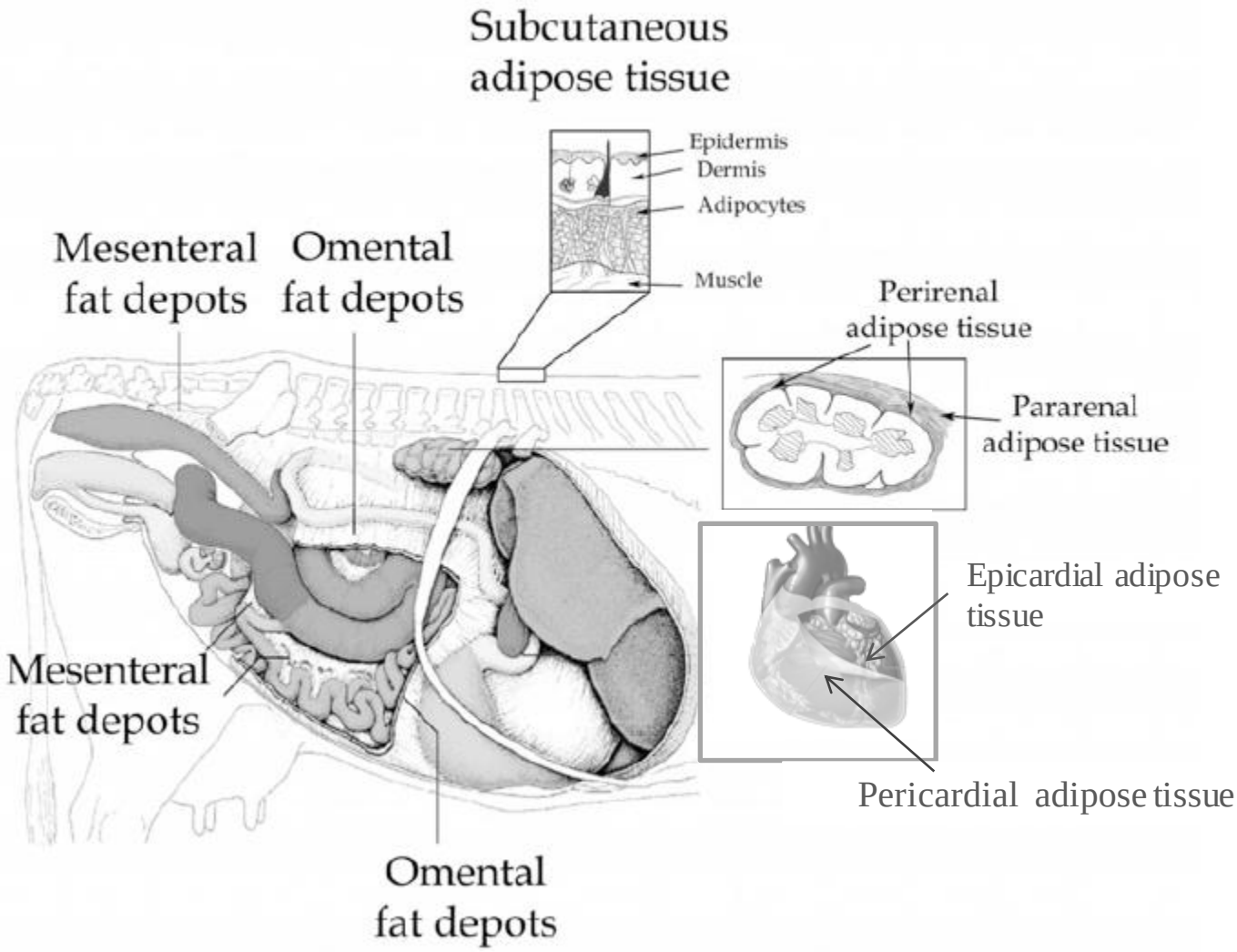


Pro-inflammatory and **anti-inflammatory** adipokines and **acute phase proteins**

Pro-mitotic and pro-angiogenetic adipokines

Modified from TILG & MOSCHEN (2006) and DENG & SCHERER (2010)

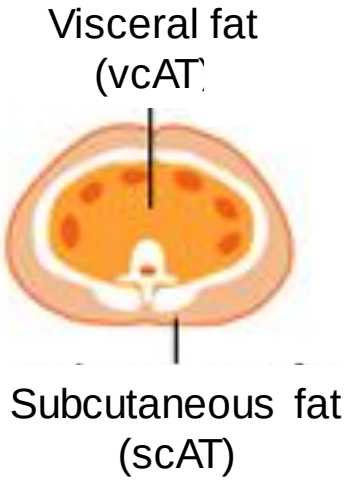
Adipose tissue, subcutaneous and visceral locations



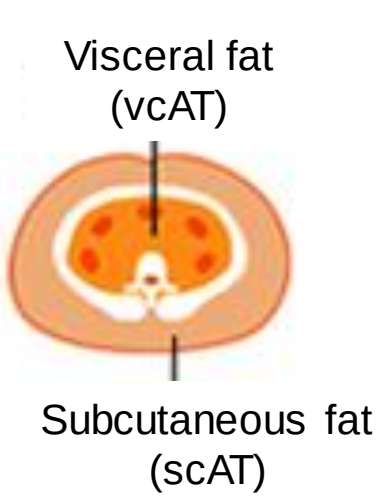
Modified from SAUERWEIN, BENDIXEN, RESTELLI & CECILIANI, *Curr Protein Pept Sci. 2014*

Different fat depots contribute differently to metabolism: Subcutaneous vs. visceral fat

Apple shaped obesity



Pear shaped obesity



A concern also for dairy cows ?

Capacity to form adipocytes:	scAT > vcAT
Vascularity:	scAT > vcAT
Metabolic activity:	scAT < vcAT
Inflammation, cytokine production:	scAT < vcAT

Different fat depots contribute differently to metabolism: Subcutaneous vs. visceral fat

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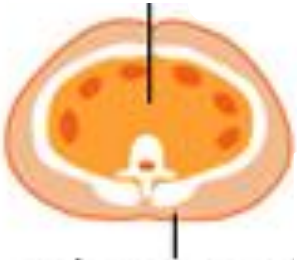

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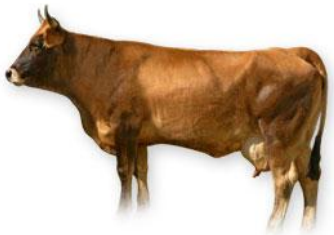

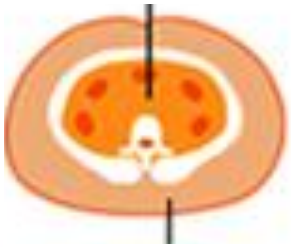

A concern also for dairy cows ?

Capacity to form adipocytes:	scAT > vcAT
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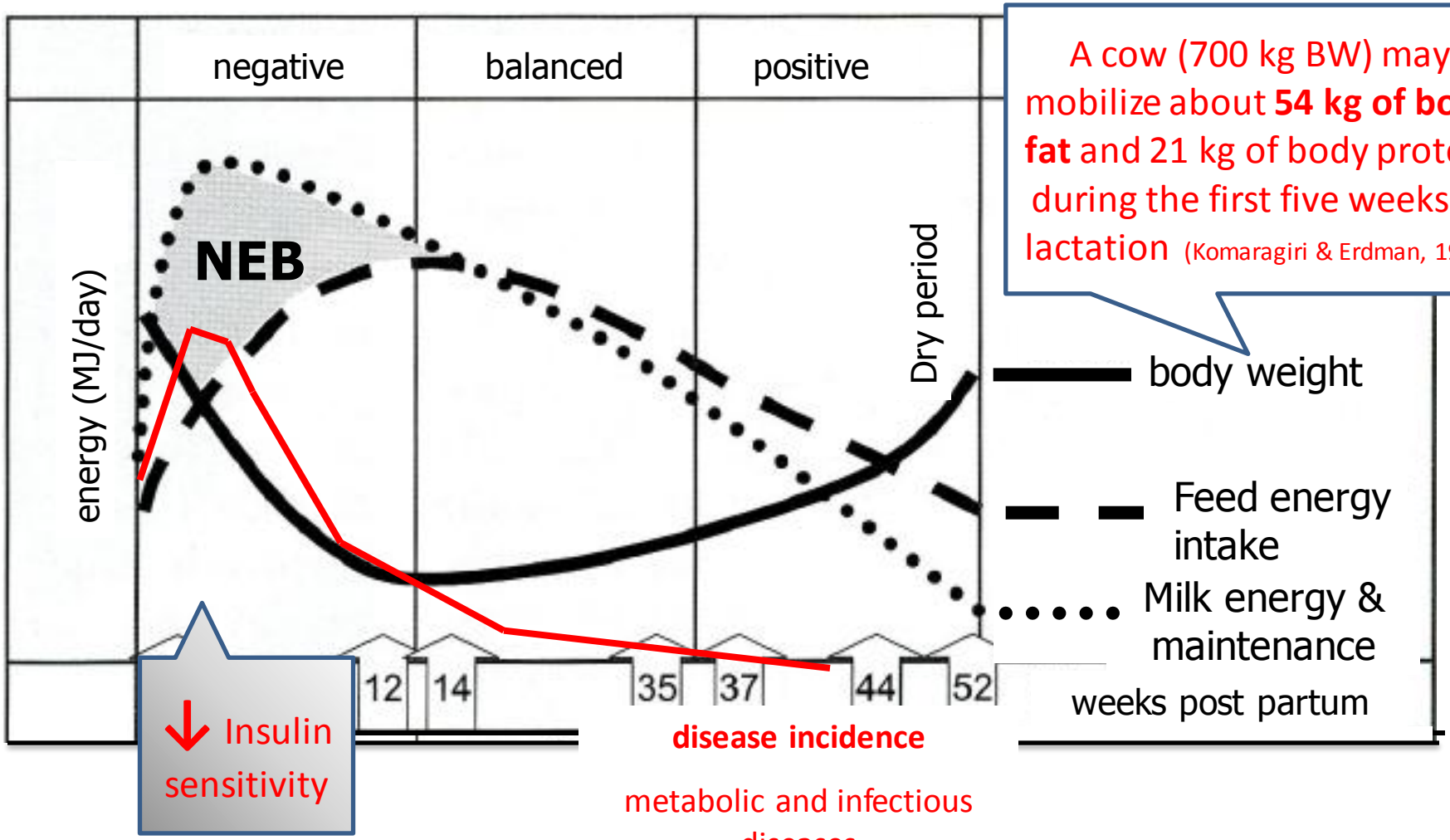
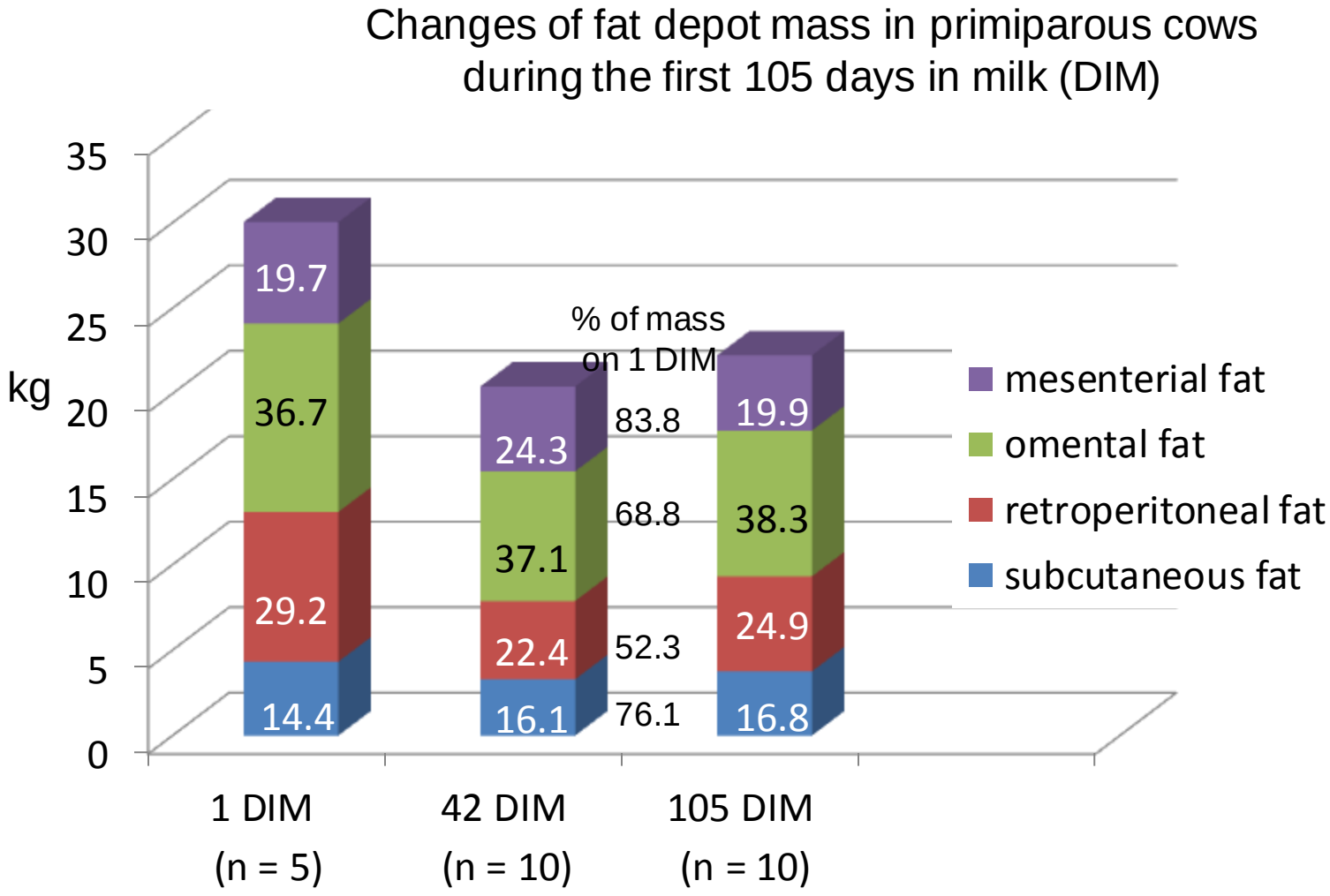
Energy status throughout lactation



A cow (700 kg BW) may mobilize about **54 kg of body fat** and 21 kg of body protein during the first five weeks of lactation (Komaragiri & Erdman, 1997)

Adapted from BUSCH et al., 2004

Visceral adiposity in dairy cows?



Modified from AKTER ET AL., 2011 *J. Dairy Sci. 95:2871*.

The main metabolic changes during the transition period:

- Lipolysis (and ketogenesis)
- reduced insulin secretion
- reduced insulin sensitivity

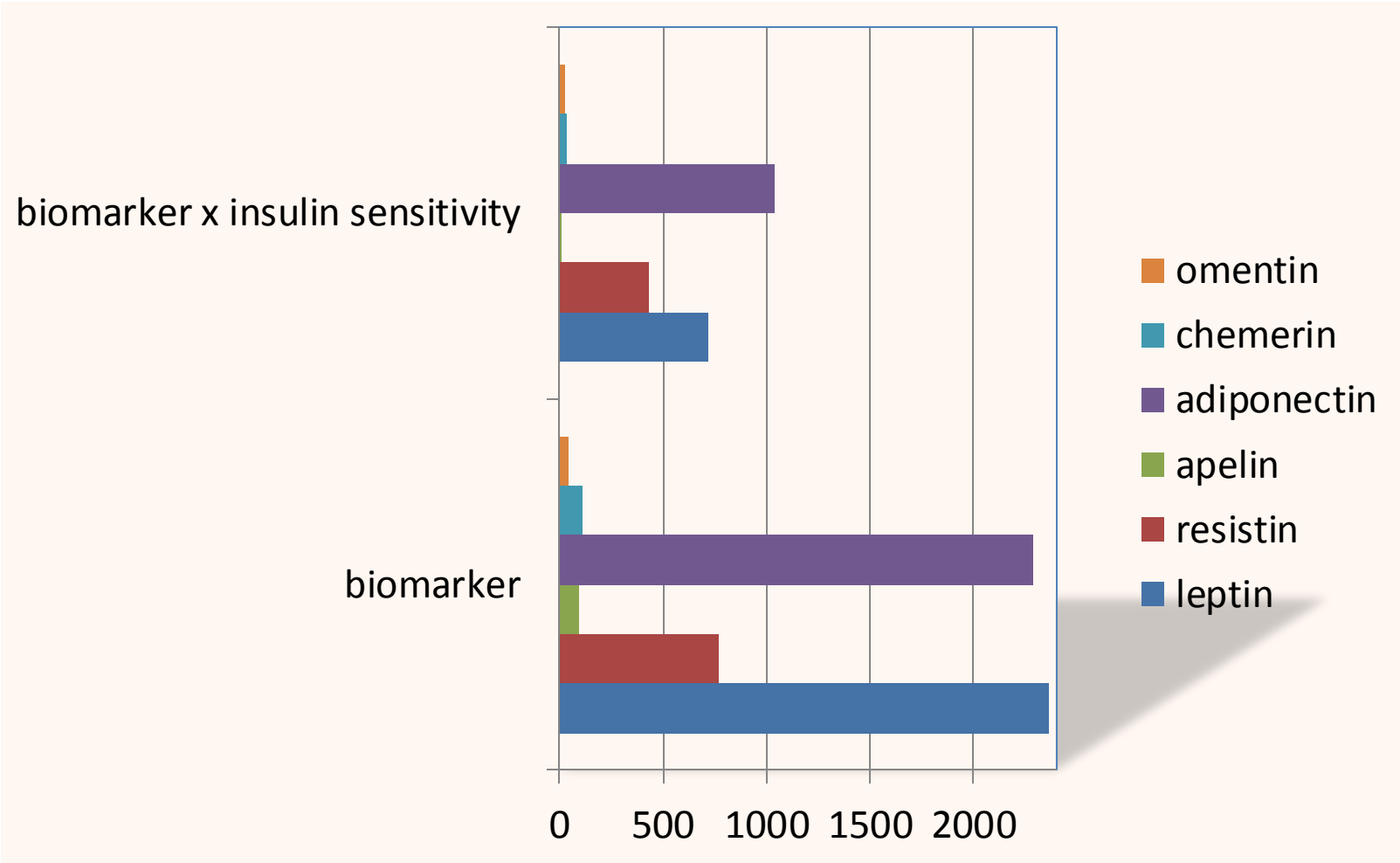
Adipokines particularly related to these metabolic changes:

- concerning changes in fat mass and size: **probably all**
- concerning insulin sensitivity: Adiponectin, leptin, and others…
- Concerning inflammation typical for the peripartal period: inflammatory cytokines*,§, Acute Phase Proteins§

* May interfere in insulin signalling
§ are not exclusively adipokines but are secreted from many other tissues (same for IGF-1)

Adipokines as biomarkers

PubMed- search results (hits) for various adipokines combined with „biomarker“ or „biomarker and insulin sensitivity“



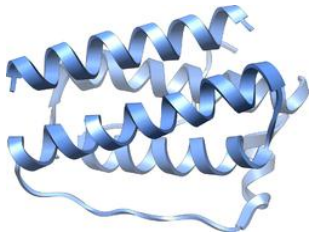
Adipokines with (almost) exclusive expression in adipose tissue

Analytical accessibility of bovine adipokines at the level of the protein

(criteria of validity published in peer reviewed journals):

- **Leptin: RIA**: DELAVAUD et al. 2000, ERHARDT et al., 2001; **ELISA**: SAUERWEIN et al., 2004
- **Resistin**: **ELISA** (distributed by Euromedex, France; supplier: USCNLife, only CV data for validity)
- **Apelin: ELISA** for bioactive fragment ,100 % species homologue (Phoenix Pharmaceuticals, USA)
- **Adiponectin: ELISA**: MIELENZ et al., 2013

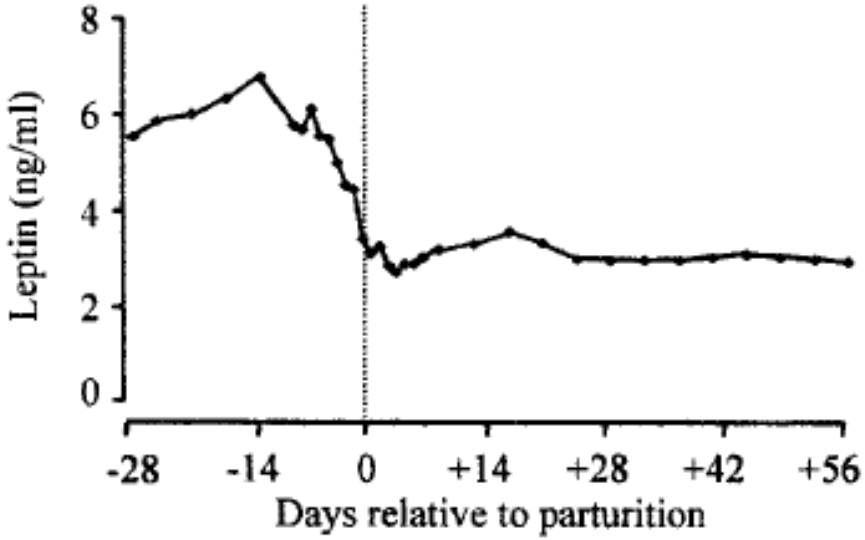
Leptin



Structure: 18 kDa protein, 167 amino acids
similar to proinflammatory helical cytokines (e.g. IL-2, IL-6)

Circulating concentrations : positively correlated with body fat content
range commonly observed in dairy cows ~ 2 – 8 ng/mL

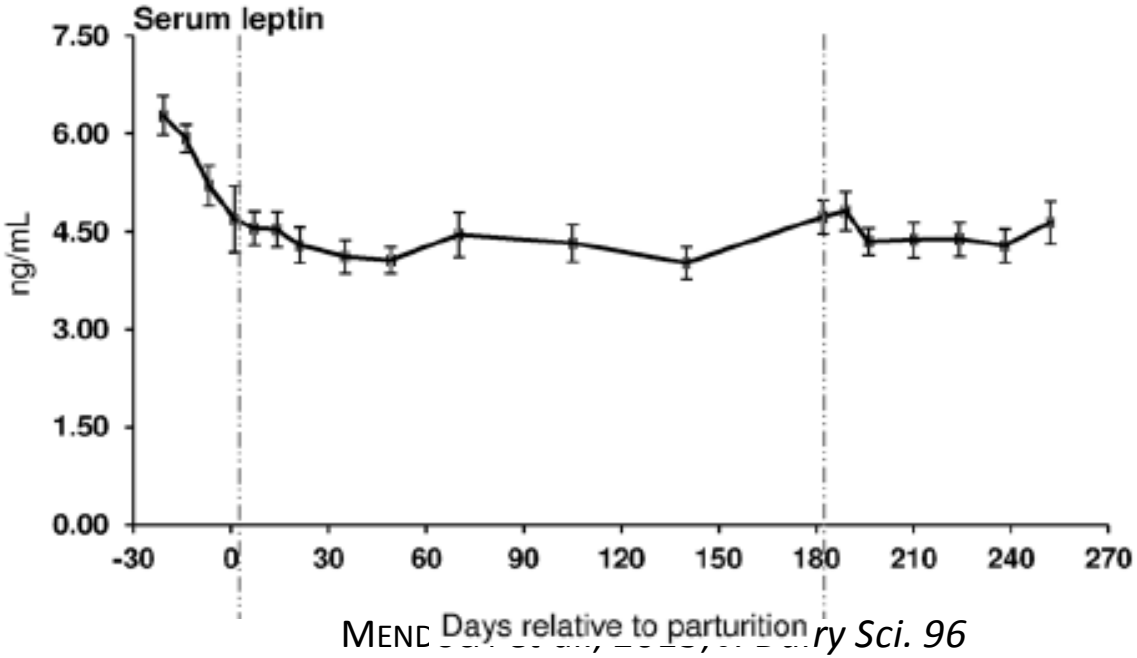
Physiological changes of the circulating concentrations during the **transition period**



BLOCK et al., 2001, *J. Endodocrinol. 171*

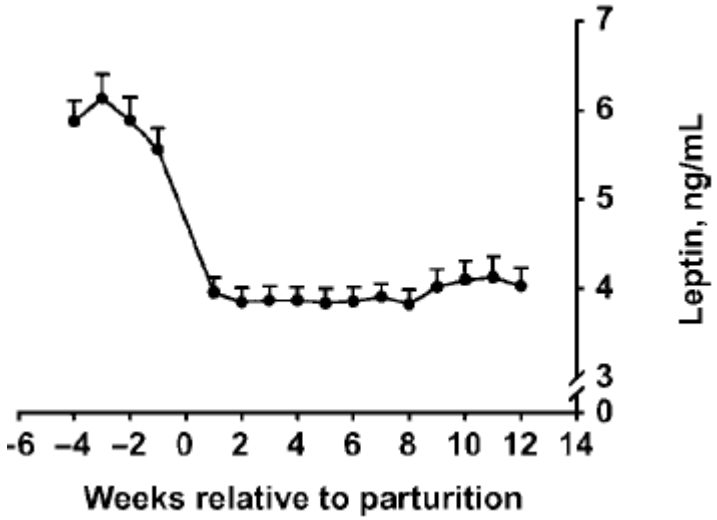
Leptin

Physiological changes of the circulating concentrations during the transition period



MENC [illegible] *ry Sci. 96*

SAREMI et al., 2014; *PLoS ONE, 9*



HACHENBERG et al., 2007, *J. Anim. Sci. 85*

Leptin

- Leptin as biomarker for metabolic health?

Table 2. Criteria of classification and variables affected in considering the earliest possible time of analysis (t) and the effort required for analysis (e)

Criterion of classification (time relative to parturition), and groups (threshold): group size (n)[1]	t[2]	e[2]	Time-courses of variables found affected[3]			
			Variable	*P*-value	ok	ltd
BCS (1 to 2 wk AP)[4]	+++	0	Leptin	0.007	↓	↑
BCS-ltd (≥3.5): 6						
BCS-ok (<3.5): 25						
Δ BCS (2 wk AP to wk 4 PP)[4]	–	1	Leptin	0.017	↑	↓
ΔBCS-ltd (≥0.5): 8			IGF-I	0.001	↑	↓
ΔBCS-ok (<0.5): 23						
β-hydroxybutyrate (wk 2 PP)	+	1	NEFA	0.001	↓	↑
BHB-ltd (≥1,200 m*M*): 14						
BHB-ok (<1,200 m*M*): 24						
NEFA (wk 1 PP)	++	1	NEFA	<0.001	↓	↑
NEFA-ltd (≥0.5 m*M*): 17			IGF-I	<0.001	↑	↓
NEFA-ok (<0.5 m*M*): 21						
IGF-I (wk 1 PP)	++	1	IGF-I	<0.001	↑	↓
IGF-I-ltd (<39 ng/mL): 19			NEFA	<0.001	↓	↑
IGF-I-ok (≥39 ng/mL): 18			Leptin	0.022	↑	↓
IGF-I-% (wk 1 AP to wk 1 PP)	++	2	IGF-I	<0.001	↑	↓
IGF-I-%-ltd (<67.9%):18			NEFA	0.003	↓	↑
IGF-I-%-ok (≥67.9%):19						
Leptin (wk 1 PP)	++	1	Leptin	<0.001	↑	↓
Leptin-ok (≥4.1 ng/mL): 19			IGF-I	0.014	↑	↓
Leptin-ltd (<4.1 ng/mL): 18						

[1]The entire time-course of the blood concentrations of the different variables was compared between each of the -ok and the -ltd groups (fixed effect); only those variables for which significant differences between the groups were found are shown.

[2]The effort required for analysis (e) and the earliest possible time of analysis (t) for the different parameters were considered and rated as follows: t, +++ = determination at wk 2-1 antepartum (AP); ++ = determination at wk 1 postpartum (PP); + = determination at wk 2 PP; and – = determination at wk 3 PP; e, 0 = no blood sample required; 1 = 1 blood sample required; and 2 = 2 blood samples required.

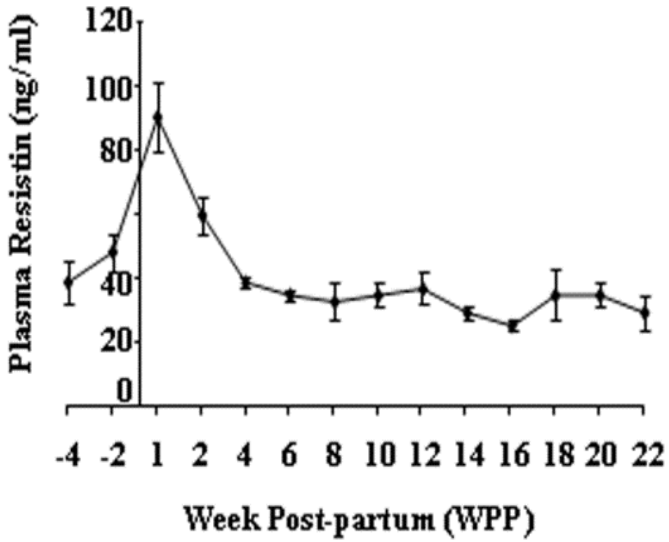
[3]↑ = greater values for the variable than the other group; and ↓ = lower values than the other group.

Resistin

Structure: cystein-rich 109 amino acid poypeptide,
circulates mainly as hexamer

Circulating concentrations :

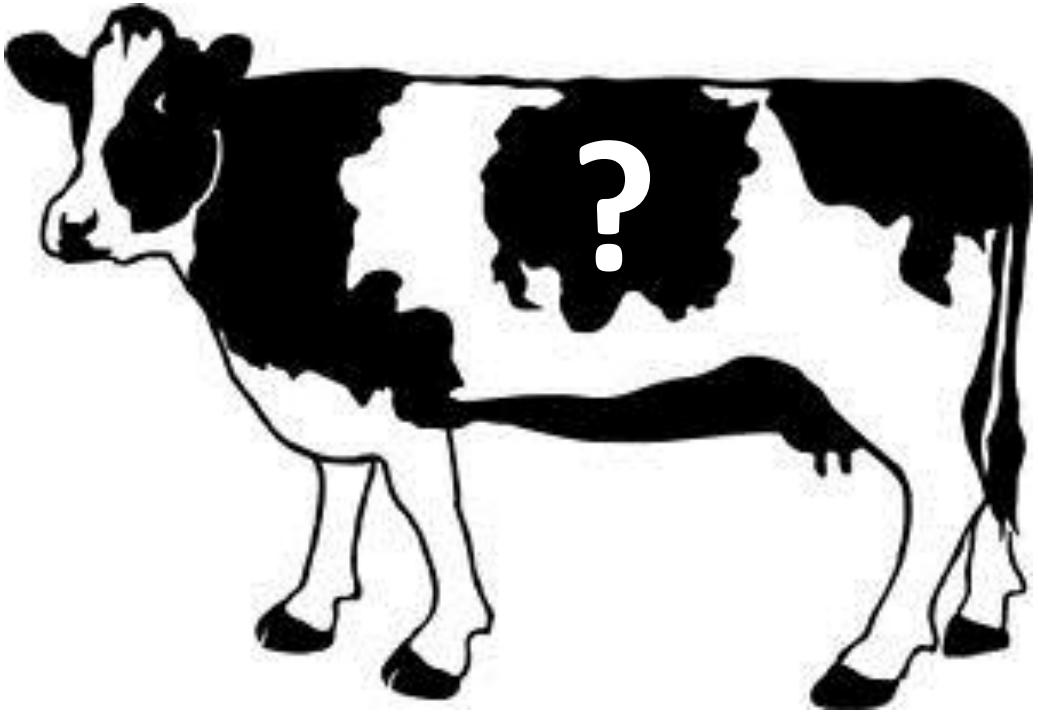
positively correlated with body fat content
positively correlated with insulin resistance
range reported for dairy cows 20 – 100 ng/



REVERCHON et al. 2014, *PLoS One.27;9*

Resistin

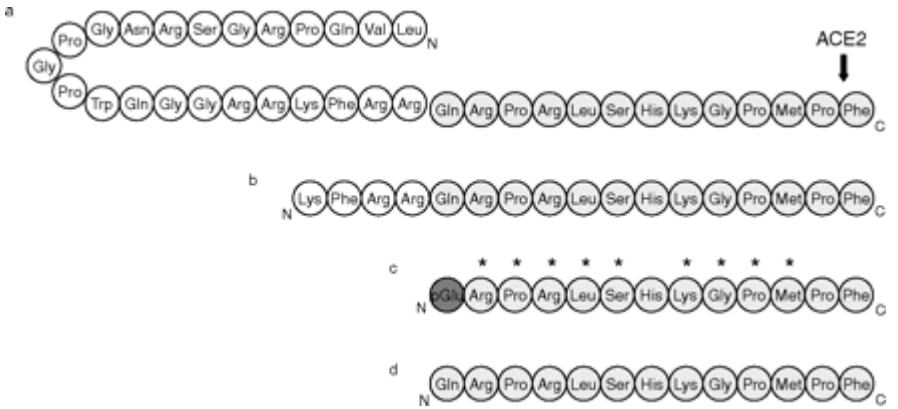
Resistin as biomarker for metabolic health?



Apelin

Structure : Apelin is synthesized as a 77 amino acid (AA) prepropeptide which is cleaved in different biologically active fragments

apelin-17, apelin-13, and [Pyr1]apelin-13 bind the G protein-coupled receptor APJ with different affinities



apelin-36

apelin-17

(Pyr1)-apelin-13

apelin-13

from PITKIN et al., 2010, *Pharmacol. Rev. 62*

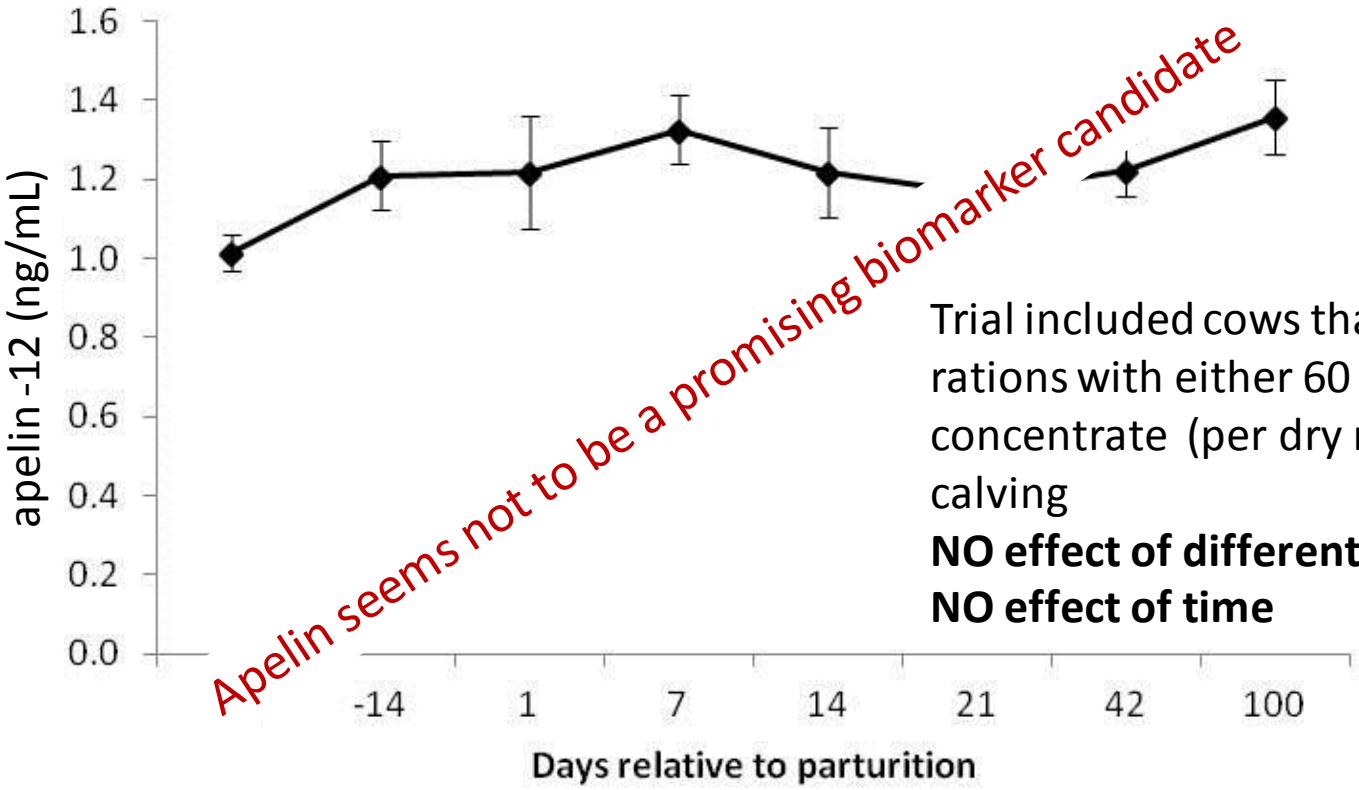
Circulating concentrations: positive correlation with body fat,

Increased insulin levels rather than adiposity is the major determinant of apelin in mice (Boucher et al., 2005)

range in dairy cows: ~ 1 ng/mL

Apelin

Physioplogical changes of the apelin serum concentrations during the transition period



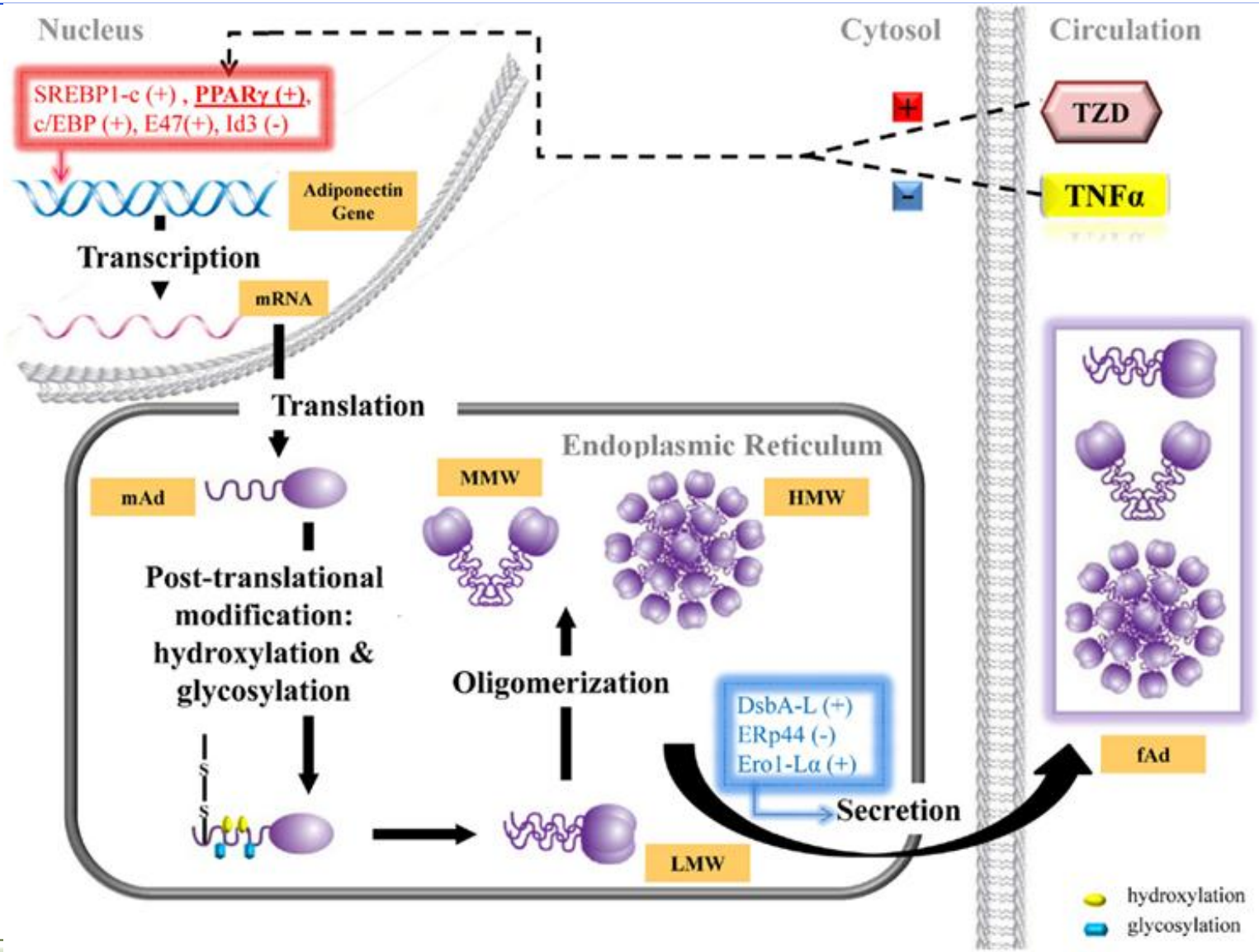
Trial included cows that were fed rations with either 60 or 30% concentrate (per dry matter) before calving

NO effect of differential feeding

NO effect of time

WEBER et al., 2014; *J. Anim Sci., 92/J. Dairy Sci, 97, p 710*

Adiponectin



From: DADSON et al., 2011, *Front. Endocrin.* 2

Adiponectin

Circulating concentrations :

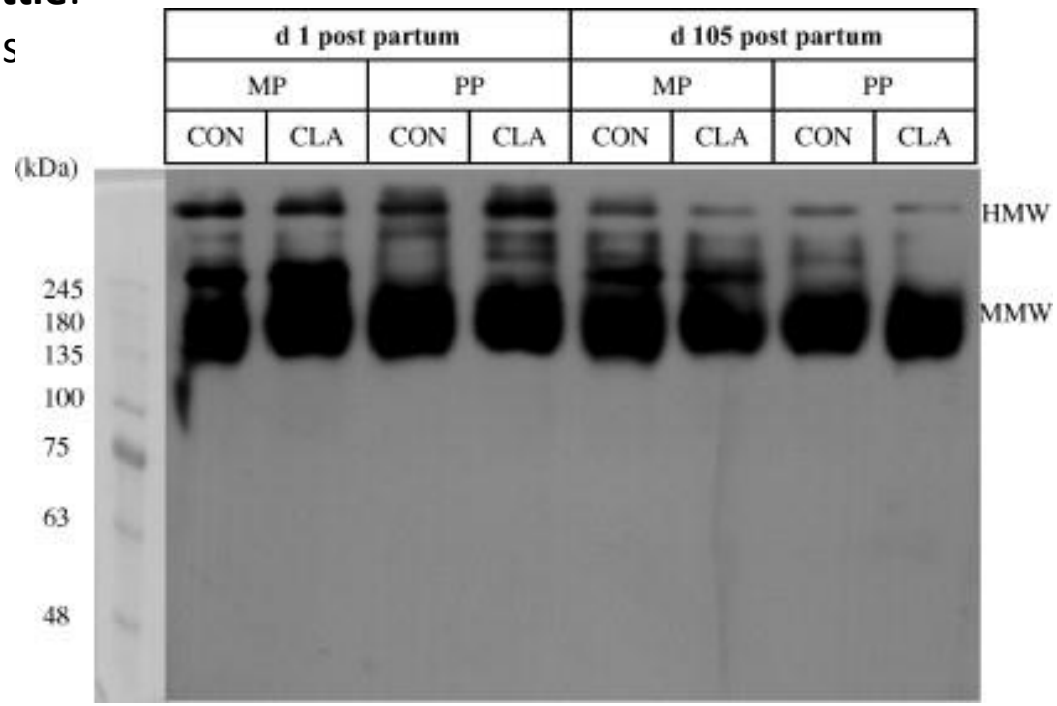
negatively correlated with body fat content

positively correlated with insulin sensitivity

range reported for dairy cows 10 – 50 **µg/mL !**

Circulating molecular weight forms in cattle:

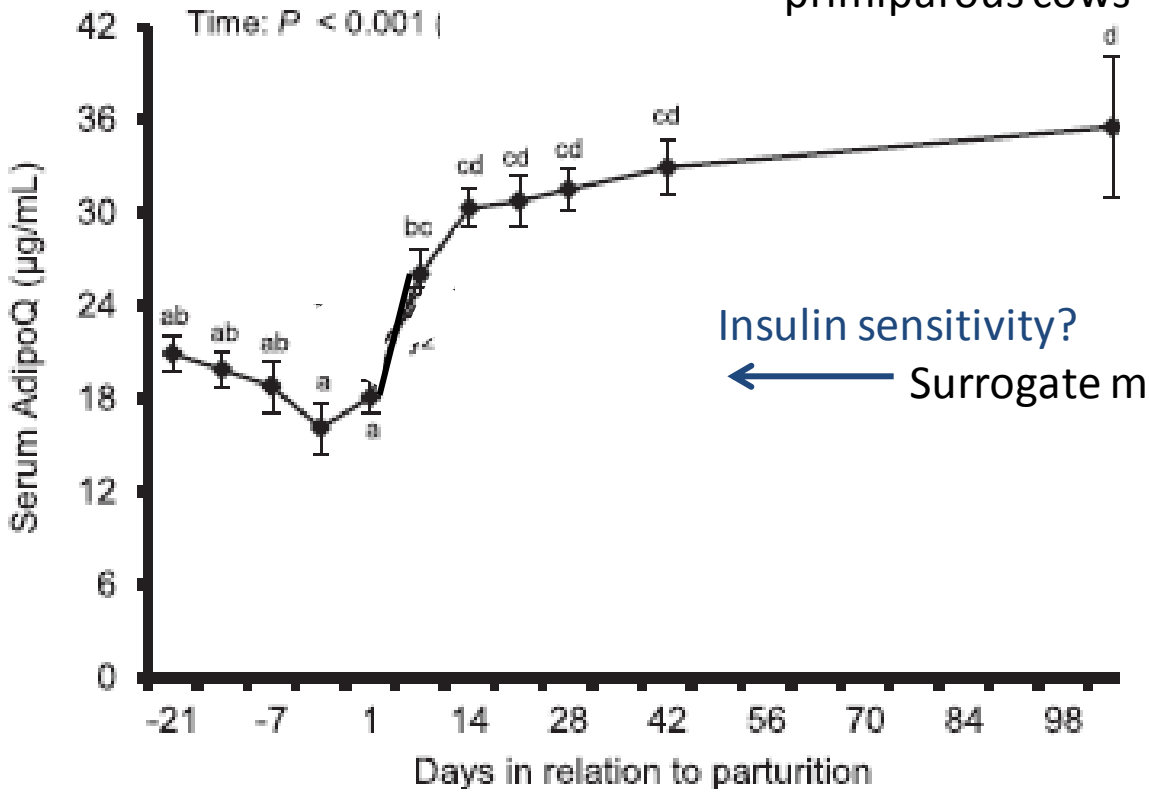
largely unaffected by physiological s
(sex, age, reproductive state)



SINGH et al., 2014, *Gen Comp Endocrinol. 198*

Adiponectin concentrations during the transition period

- primiparous cows -



Insulin sensitivity?

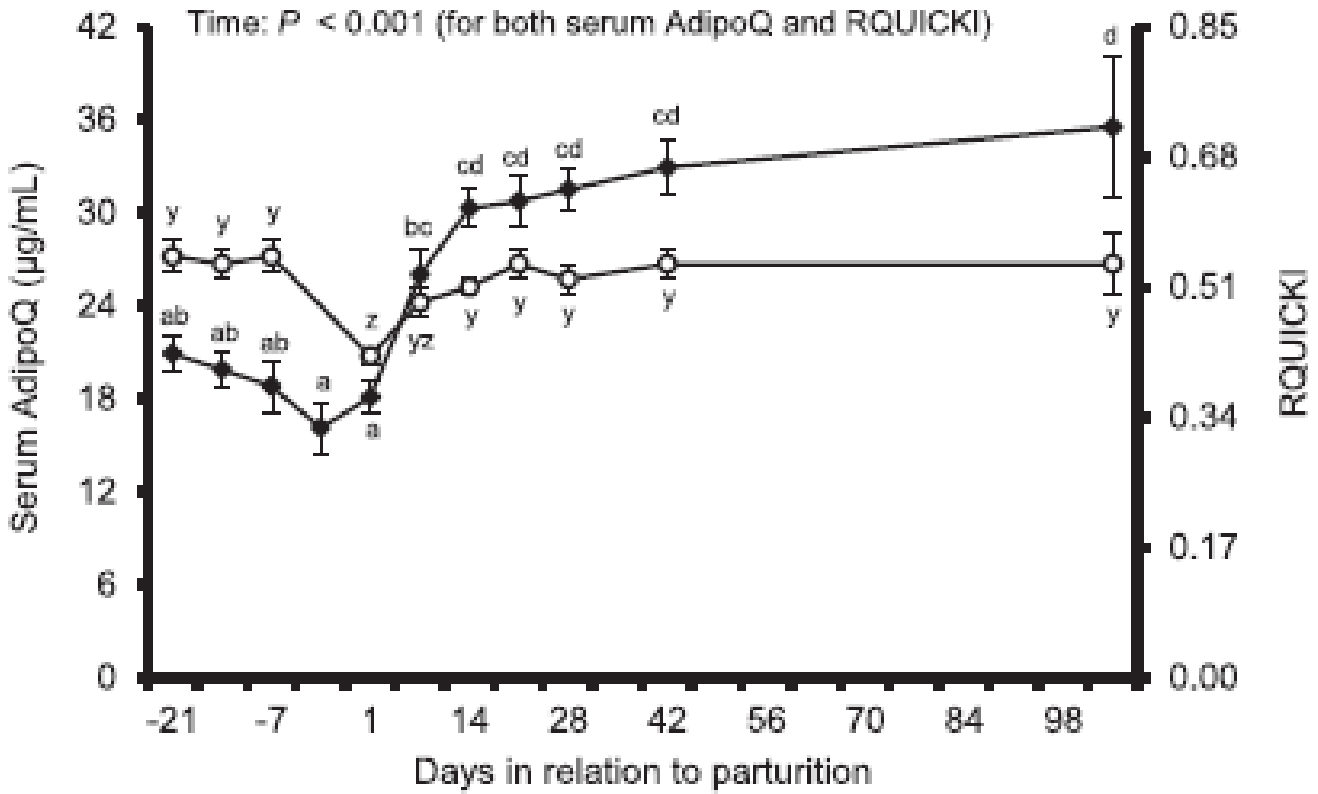
← Surrogate marker: RQUICKI*

SINGH et al., 2014, *Domest. Anim. Endocrinol. 47*

*RQUICKI = 1/[log (glucose, mg/dL) + log (insulin, µU/mL) + log (NEFA, mM)]
(HOLTENIUS & HOLTENIUS, 2007, *Acta Vet. Scand. 49)*

Adiponectin concentrations during the transition period

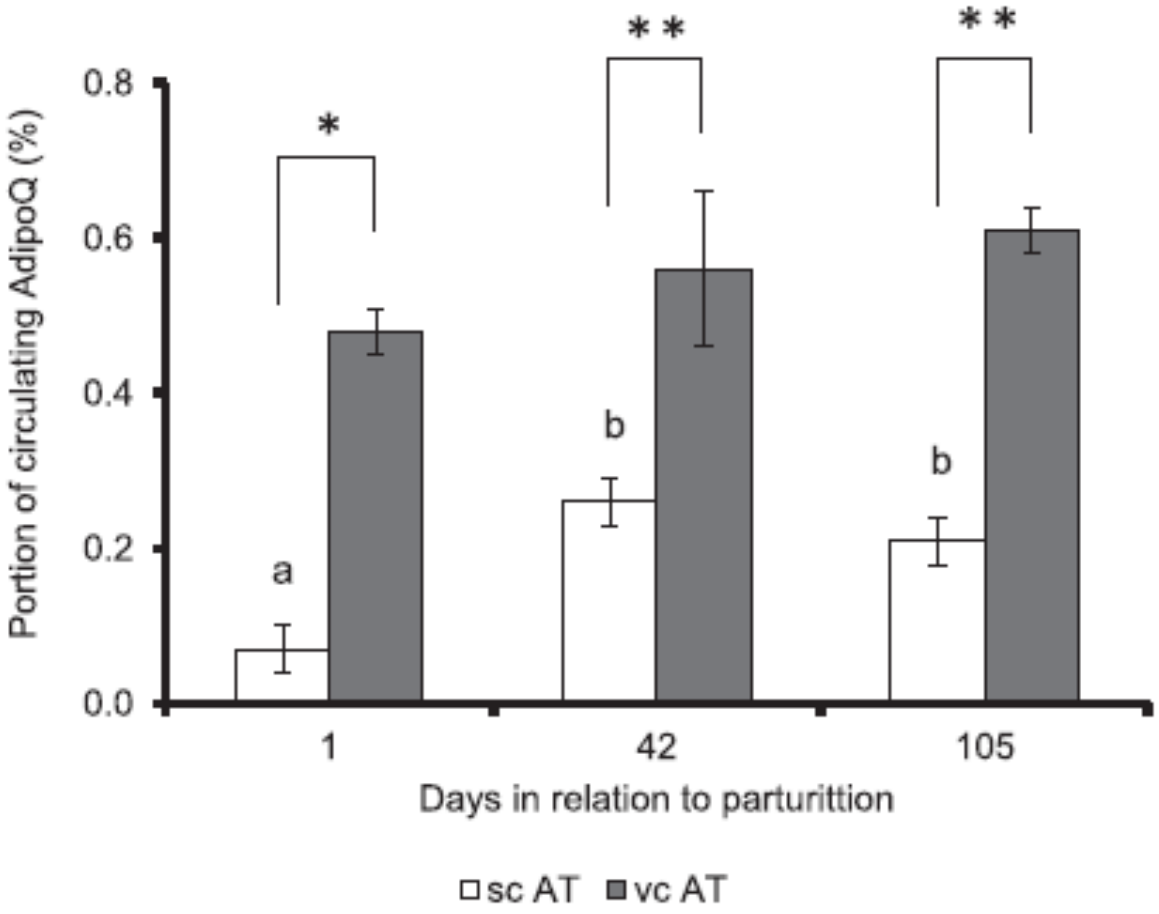
- primiparous cows -



SINGH et al., 2014, *Domest. Anim. Endocrinol. 47*

Contribution of visceral versus subcutaneous fat depots to circulating adiponectin

- primiparous cows -



SINGH et al., 2014, *Domest. Anim. Endocrinol. 47*

Contribution of visceral versus subcutaneous fat depots to circulating adiponectin

s cows -

Multiple linear regression analyses of the relationship between AT depot measures with serum AdipoQ concentration.

Predictor variable	Standardized β coefficient	*P*	Adjusted R^2
Model 1			0.654
Log retroperitoneal AdipoQ, ng/g tissue	0.650	<0.001	
Log tail-head AdipoQ, ng/g tissue	0.366	0.009	
Model 2			0.608
Retroperitoneal tissue mass, kg	−0.564	<0.001	
Log omental total AdipoQ, μg	0.515	0.001	

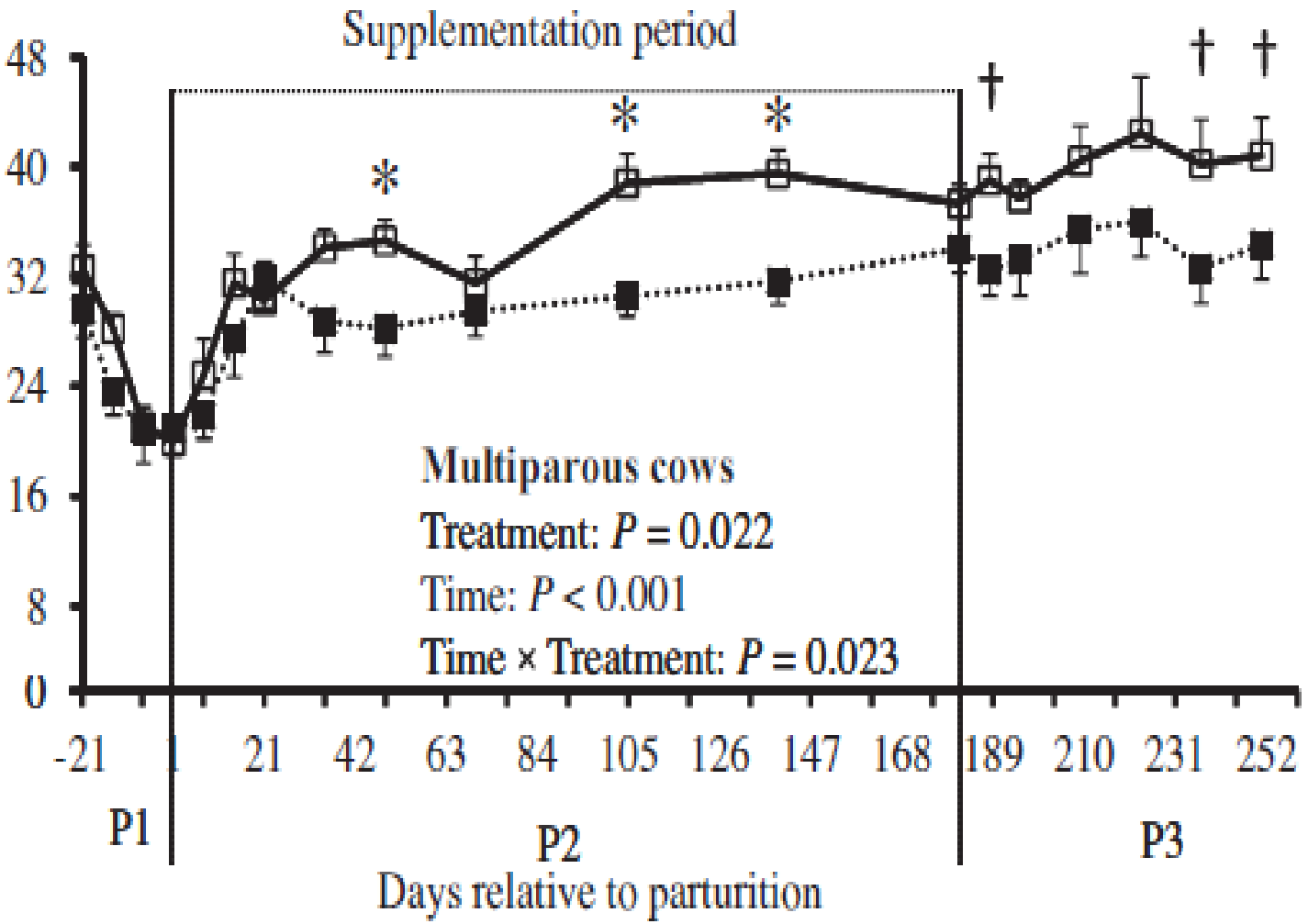
Abbreviations: AdipoQ, adiponectin; AT, adipose tissue.
Parameters identified as significant independent predictors for serum adiponectin in dairy cows are presented.
For model 1 AdipoQ concentrations (ng/mg tissue) in each individual AT (mesenterial, omental, retroperitoneal, sternum, tail-head, and withers AT) were included, whereas for model 2 retroperitoneal tissue mass (kg), retroperitoneal adipocyte size (μm^2), wither adipocyte size (μm^2), and log omental total AdipoQ (μg) were included because of their strong relationship with serum AdipoQ ($r \geq 0.55$).

Portion of circulating AdipoQ (%)

SINGH et al., 2014, *Domest. Anim. Endocrinol. 47*

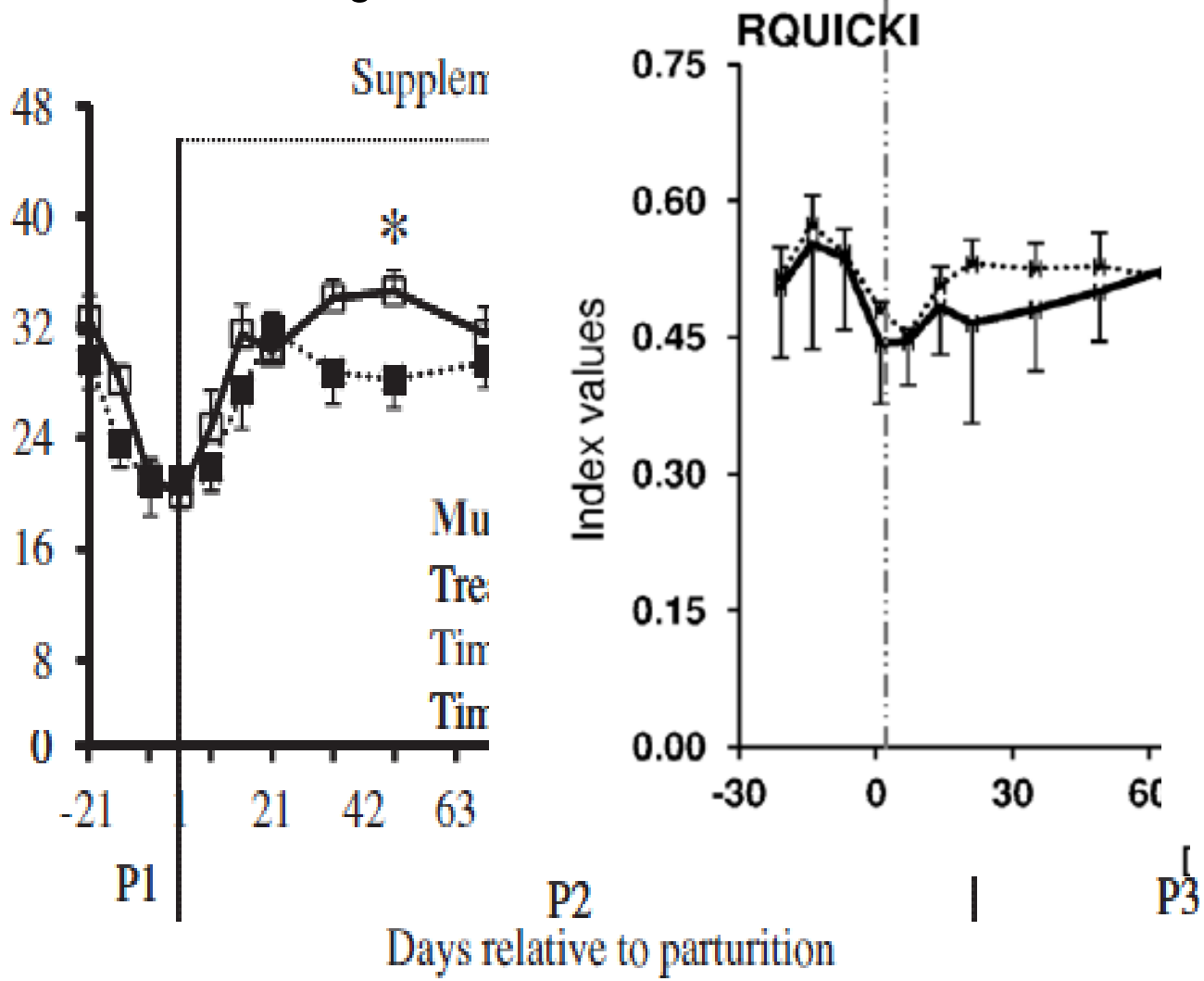
Adiponectin

Change of the circulating concentrations during the transition period in dairy cows:

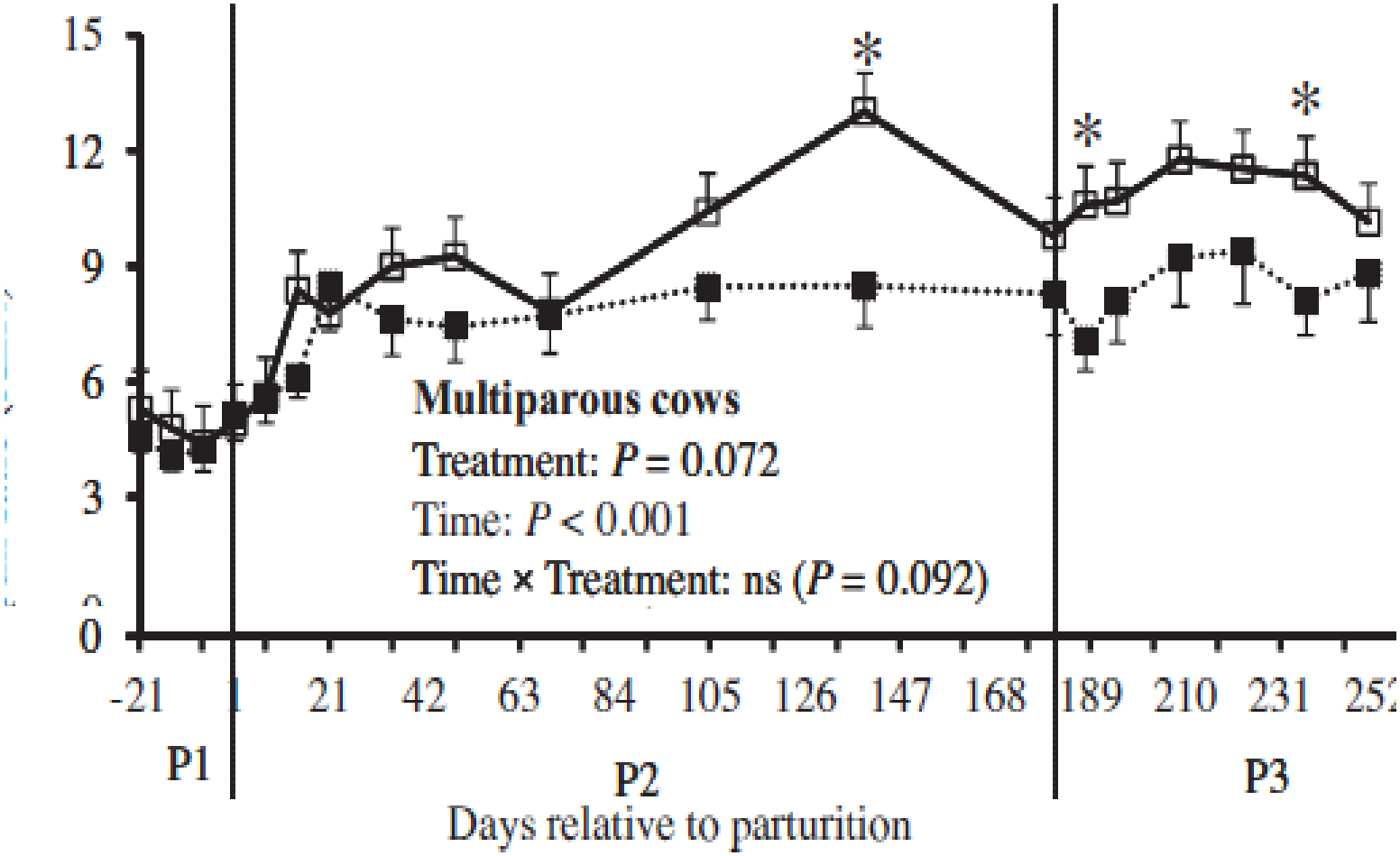


Adiponectin

Change of the circulating concentrations during the transition period in dairy cows:



Adiponectin : leptin ratio



Summary and critical assessment I

Adiponectin seems the most promising candidate, because

- it was correlated with RQUICKI BUT: r = 0.126 – 0.485, RQUICKI is a surrogate marker
- it revealed treatment effects supported by RQUICKI data BUT: relevance unknown
- visceral contribution > subcutaneous BUT: database limited to one slaughter experiment in primiparous cows

Impaired insulin regulation of energy metabolism = etiologic key component for metabolic diseases typically for the transition period

Adiponectin holds promise as an indirect biomarker of insulin sensitivity

Adipokines as biomarkers for metabolic health?

General sceptisism:

- differences are relatively small
- predictive value for metabolic diseases has not yet been tested,
- data on sensitivity-specificity are lacking,
- analytical effort is considerable,
- best time of sample collection remains open
- reference values are not defined
- No perspective for non-invasive sampling (?...adiponectin in saliva…?)

HOWEVER,

- DairyCare combines different aspects, different expertises
- The combination per se holds promise
- Even though we might not come up with „patent solutions“

The welfare issue requires our efforts, we need to do what we can

Acknowledgements

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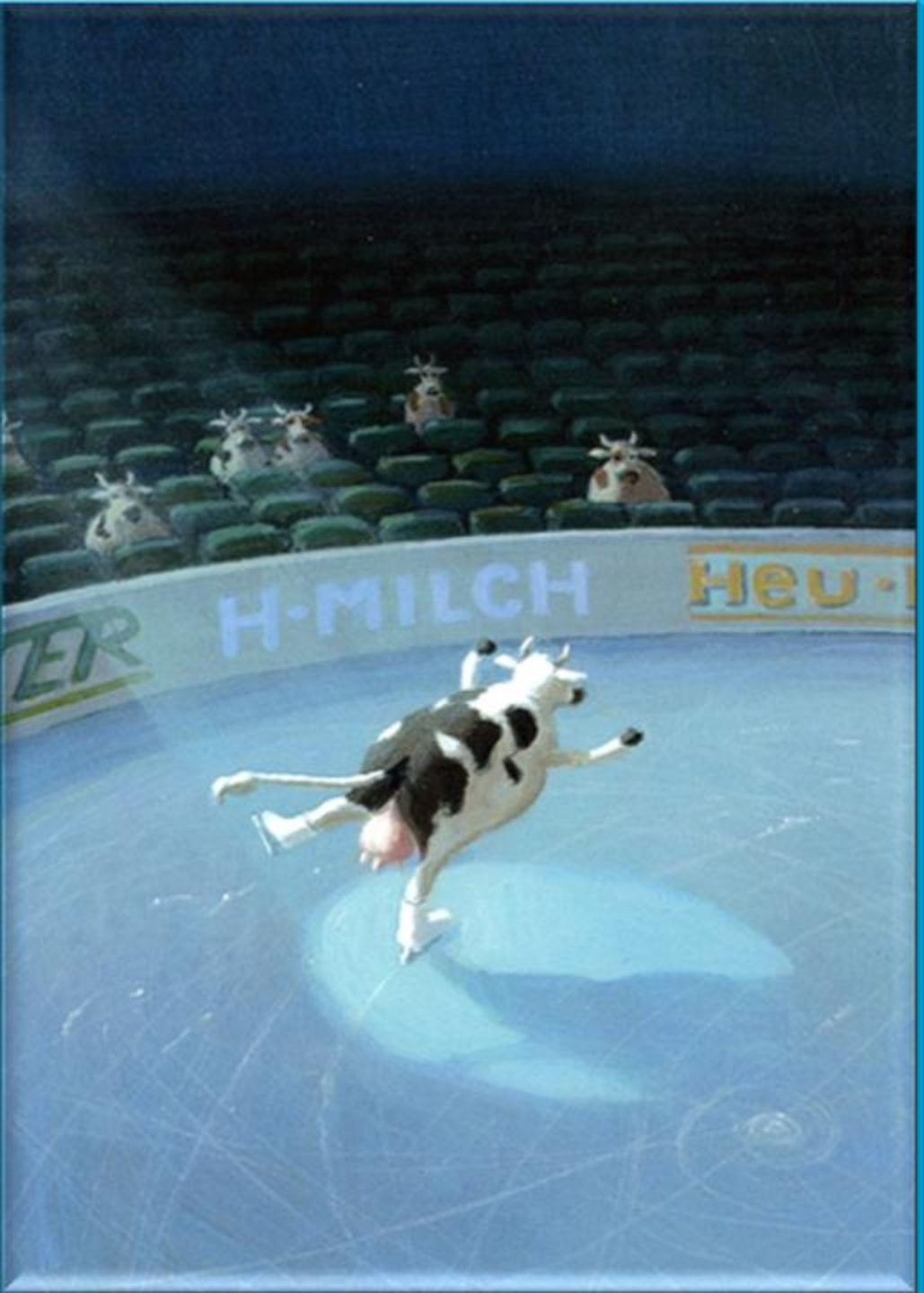

…keeping the balance….

Thank you for your attention