Hannelore Daniel

# „Perspektiven für die Entwicklung von Zusatzstoffen“

## *Funktionalität und Health Claims*

Schaumann-Stiftung, Lübeck, 2012

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# „Perspektiven für die Entwicklung von Zusatzstoffen“

## *Funktionalität und Health Claims*

Trends | Health claims | Verfahren | Beispiele | Perspektive

Schaumann-Stiftung, Lübeck, 2012

# Der Lebensmittelsektor im ZEITGEIST


Quelle: in Anlehnung an Leo A. Nefiodow: „Der sechste Kondratieff"

# Der Lebensmittelsektor im ZEITGEIST

Convenience

Gesundheit

Enjoyment

***Unbedenklichkeit***

Conv

WEI

Genuß

**Die WERTE-ORDNUNG des Konsumenten**

1. Schutz der Familie
2. Gesundheit und Fitness
3. Ehrlichkeit
4. Selbstverwirklichung
5. Freiheit
6. Gerechtigkeit
7. Selbstvertrauen
8. Freundschaft
9. Wissen
10. Andauernde Liebe

* 57 Personal values measured

Source: 2005 Roper Reports Worldwide

**Werte**

# Phytosterol(stanol)haltige Produkte als erste „echte“ funktionelle Lebensmittel



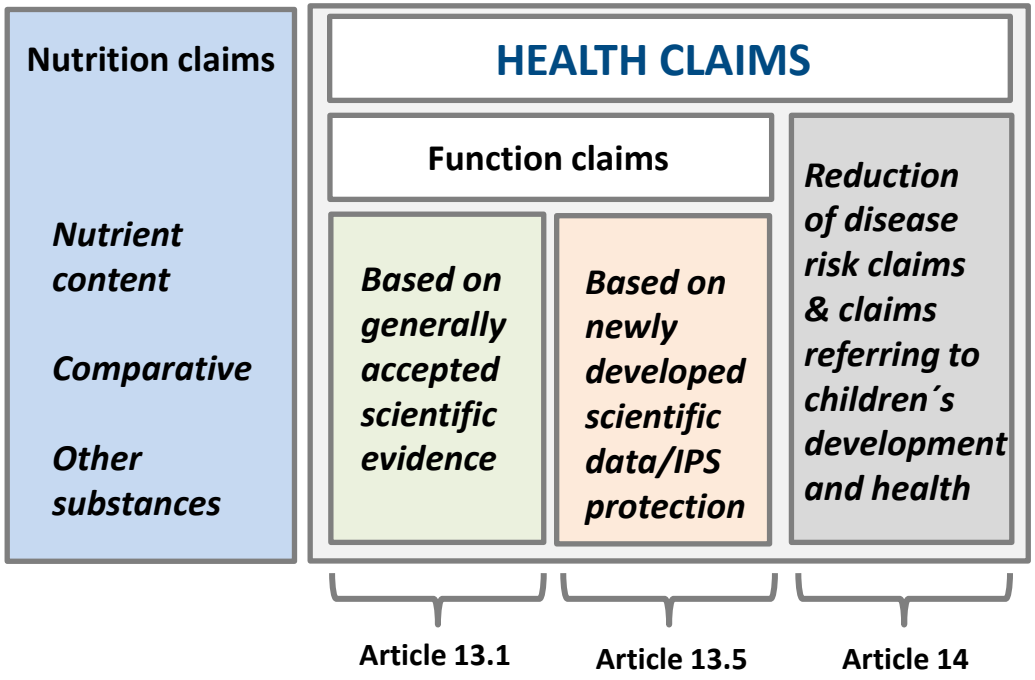
Phytosterole



Phytostanole

European Food Safety Authority

**Health claims made in relation to food products require authorisation under Regulation EC 1924/2006 before they can be used in the labelling and marketing of these products in the EU. Within the context of this authorisation procedure, EFSA's Panel on Dietetic Products, Nutrition and Allergies (NDA) is responsible for verifying the scientific substantiation of the health claims.**


Nutrition claims
Nutrient content
Comparative
Other substances
HEALTH CLAIMS
Function claims
Based on generally accepted scientific evidence
Based on newly developed scientific data/IPS protection
Reduction of disease risk claims & claims referring to children´s development and health
Article 13.1
Article 13.5
Article 14

efsa
European Food Safety Authority

**The publication of the final series of 35 evaluations is the culmination of more than three years' work by EFSA's experts. Since 2008 the Panel has assessed 2,758 food-related general function health claims to determine whether they were supported by sound scientific evidence, thereby assisting the European Commission and Member States in establishing a list of claims authorised for food. These approved claims can help European consumers to make more informed choices about their diet.**

**"EFSA's independent evaluation concluded that a considerable number of claims made on foods are backed by sound science, including claims related to a wide range of health benefits."**

European Food Safety Authority

**The outcomes of evaluations were favourable when there was sufficient evidence to support the claims. This was the case for about one in five claims reviewed, which related mainly to:**

- vitamins and minerals;
- specific dietary fibres related to blood glucose control, blood cholesterol, or weight management;
- live yoghurt cultures and lactose digestion;
- antioxidant effects of polyphenols in olive oil;
- walnuts and improved function of blood vessels;
- meal replacement and weight control;
- fatty acids and function of the heart;
- the role of a range of sugar replacers (such as xylitol and sorbitol) in maintaining tooth mineralisation or lowering the increase of blood glucose levels after meals;
- carbohydrate-electrolyte drinks/creatine and sports performance.

European Food Safety Authority

## Experts issued unfavourable opinions in cases where the information provided did not allow a relationship between the food and the claimed effect to be established. Reasons included:

- lack of information to identify the substance on which the claim is based (for example, claims on “probiotics”, or on “dietary fibre” without specifying the particular fibre);
- lack of evidence that the claimed effect is indeed beneficial to the maintenance or improvement of the functions of the body (for example, food with “antioxidant properties” and claims on renal “water elimination”);
- lack of precision regarding the health claim being made (for example, claims referring to terms such as “energy” and “vitality”, or claims on women’s health or mental energy);
- lack of human studies with reliable measures of the claimed health benefit;
- claims referring to food categories which were considered to be too broad, such as “fruits and vegetables” and “dairy products” to be linked to specific effects.

# In weighing the evidence, the Panel …..

# Wieviel Wissenschaft braucht der Sektor ?

***The world is (intends to be) evidence based !***

# Definitionen: Was ist Wissenschaft ?

**Lebens- und Weltorientierung, die auf eine spezielle (meist berufsmäßig ausgeübte) Begründungspraxis aufgebaut ist; die Tätigkeit, die das wissenschaftliche Wissen hervorbringt**
**(Meyers Enzyklopädisches Lexikon)**

**Wissenschaft fragt nach dem Warum, den Gründen und den Ursachen der Dinge**
**(Aristoteles)**

**das nach Prinzipien geordnete Ganze der Erkenntnis**
**(Kant)**

**Wissenschaft ist das, was anerkannte Wissenschaftler für Wissenschaft halten**
**(Odo Marquard)**

# Qualitätskriterien für die Wissenschaft

**Widerlegbarkeit**

**Widerspruchsfreiheit**

**Kritisierbarkeit**

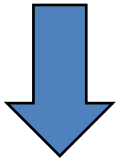
**Prüfbarkeit**

**Hypothese**

**Beschreibung der Studien**

**Beschreibung der Ergebnisse**

**Interpretation im Kontext anderer Befunde**



**Plausibilität**

**Vollmer: Was können wir wissen?, Popper: Logik der Forschung, Albert: Traktat über kritische Vernunft**

# *Totality of scientific evidence*

## Kernfragen:

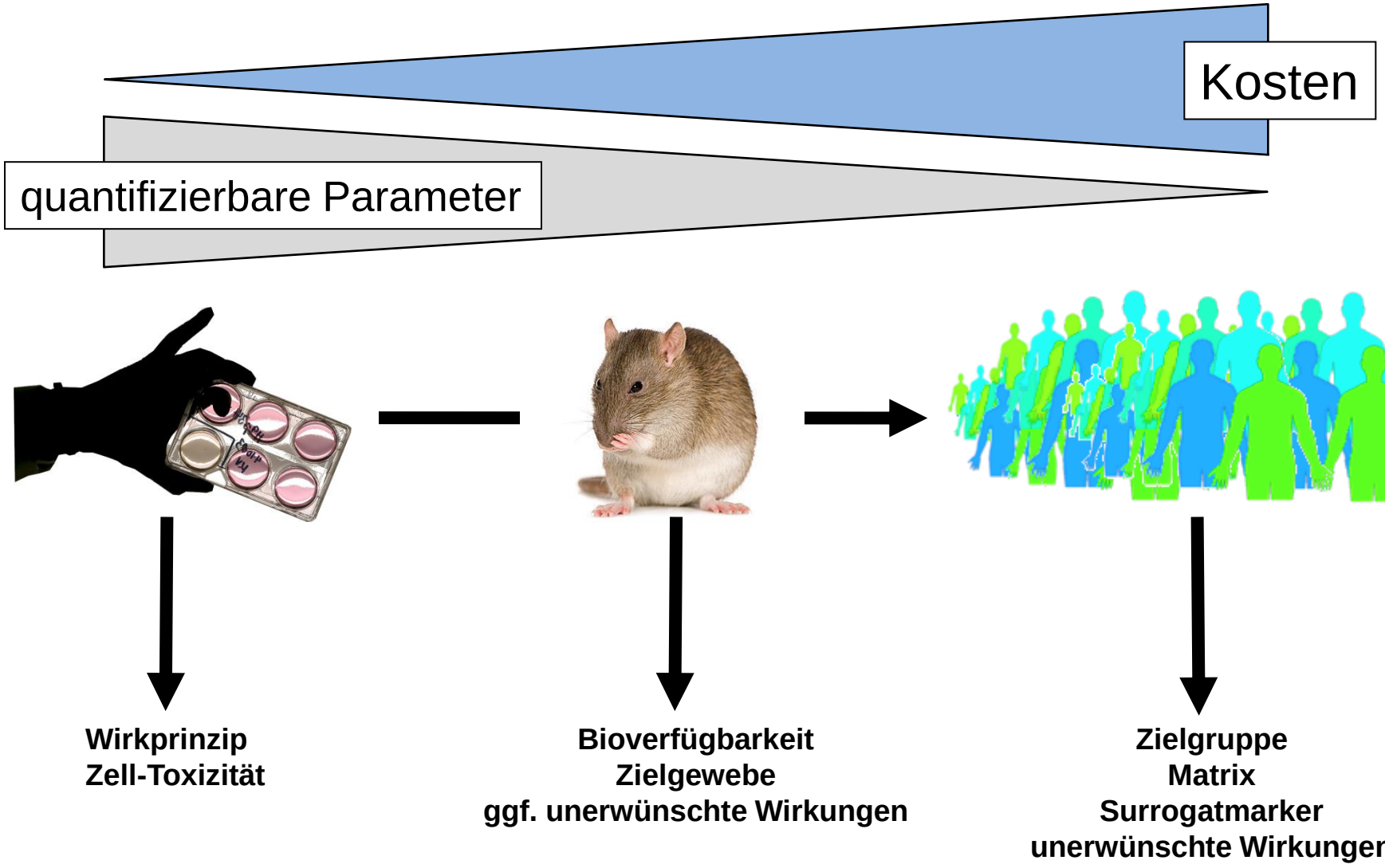
- **Ist der postulierte Wirkmechanismus plausibel und durch eine hinreichende Zahl an Studien belegt ?**
- **Gibt es eine Dosis-Wirkungsbeziehung (für den gewünschten Effekt wie für die möglichen Nebenwirkungen) ?**
- **Wie ist die Wirksamkeit in der Lebensmittelmatrix (LM-Effekt) und ist die Verzehrmenge realistisch/plausibel ?**
- **Welche Zielgruppe ist intendiert und wie ist die Wirksamkeit in der Zielgruppe (Risk-Benefit-Analyse) ?**

## *Totality of scientific evidence*

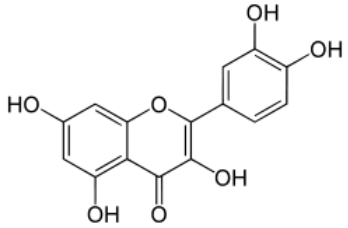
# Es ist immer *CASE by CASE*

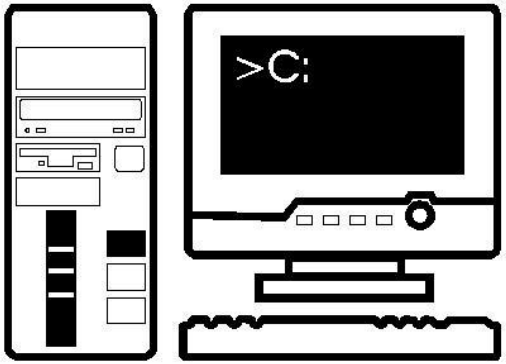
***case by molecule***
***case by biology***
***case by target group***

# *Totality of scientific evidence*



# *Totality of scientific evidence*



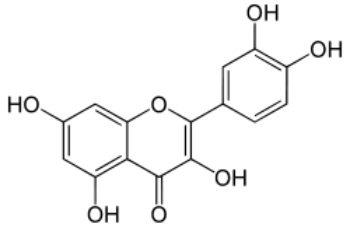

**umfassende Literatursuche**

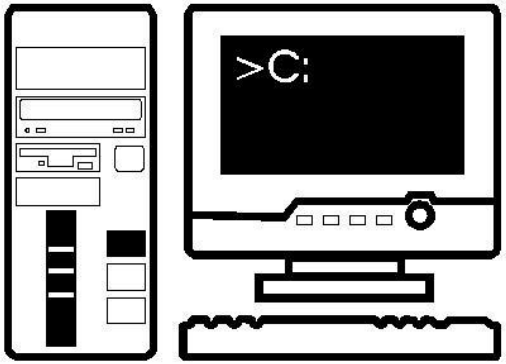
- Studien in Zellkulturen
- Studien in Modellorganismen
- Studien in Probanden

Ableitung von Parametern zur Plausibilität des Mechanismus und zur Effektgröße (stat. Signifikanz) und damit Größe der Kohorte für notwendige Humanstudien

**Wenn keine ausreichenden Informationen vorliegen, dann Substanzen mit ähnlichen physikalisch-chemischem Eigenschaften oder ähnlicher intendierter Funktionalität prüfen (Plausibilität).**

# *Totality of scientific evidence*




**umfassende Literatursuche**

- Studien in Zellkulturen
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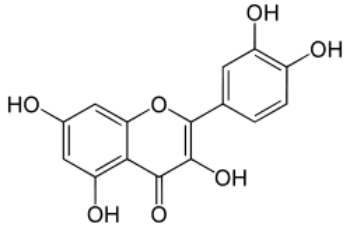
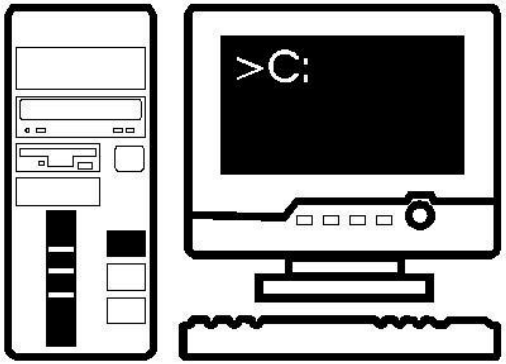
**Quercetin: 8574**
**Publikationen (2012)**

**Quercetin**
**/cell: 3902**
**/mechanism: 727**
**/mice: 862**
**/rat: 931**
**/human: 3107**

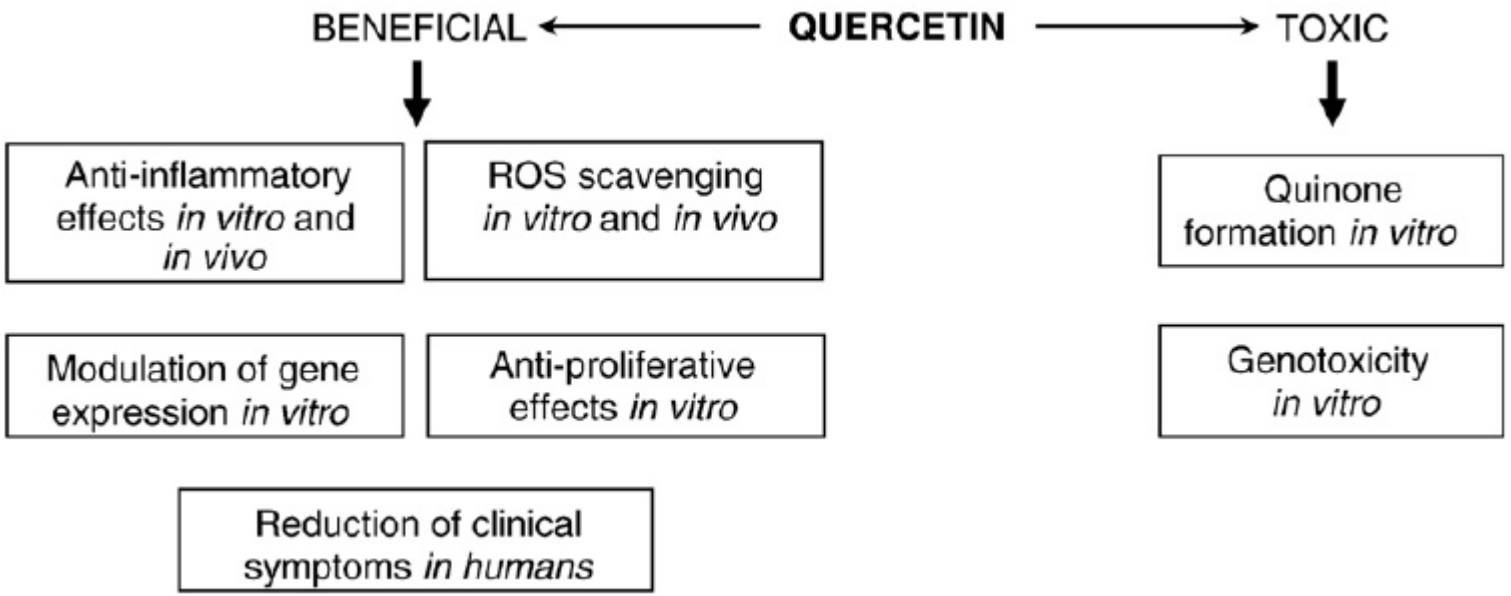
# *Totality of scientific evidence*




**umfassende Literatursuche**

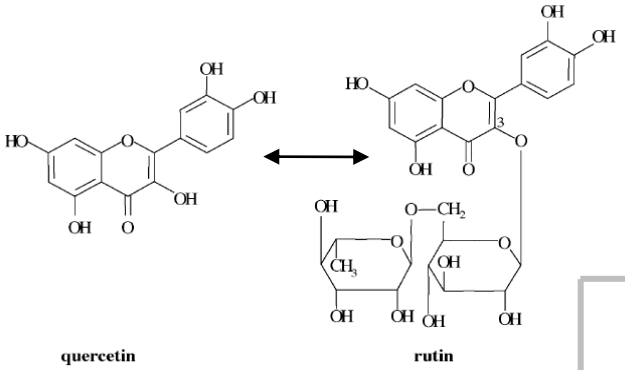
Schematic overview of the beneficial and toxic effects of quercetin *in vitro* and *in vivo*.

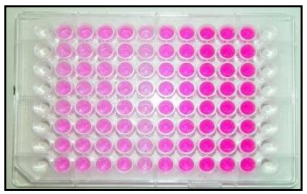


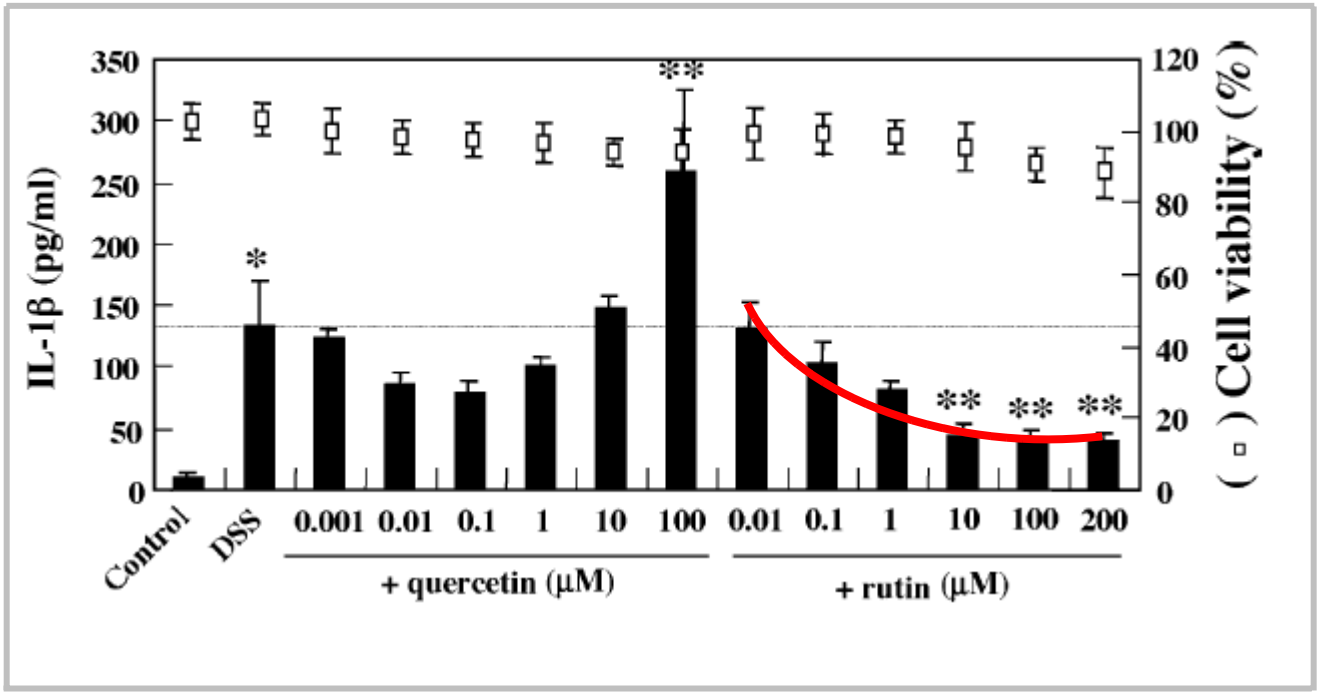

*A.W. Boots et al. / European Journal of Pharmacology 585 (2008) 325-337*

# *Totality of scientific evidence*

## Von der Plausibilität zur Dosis-Wirkungsbeziehung



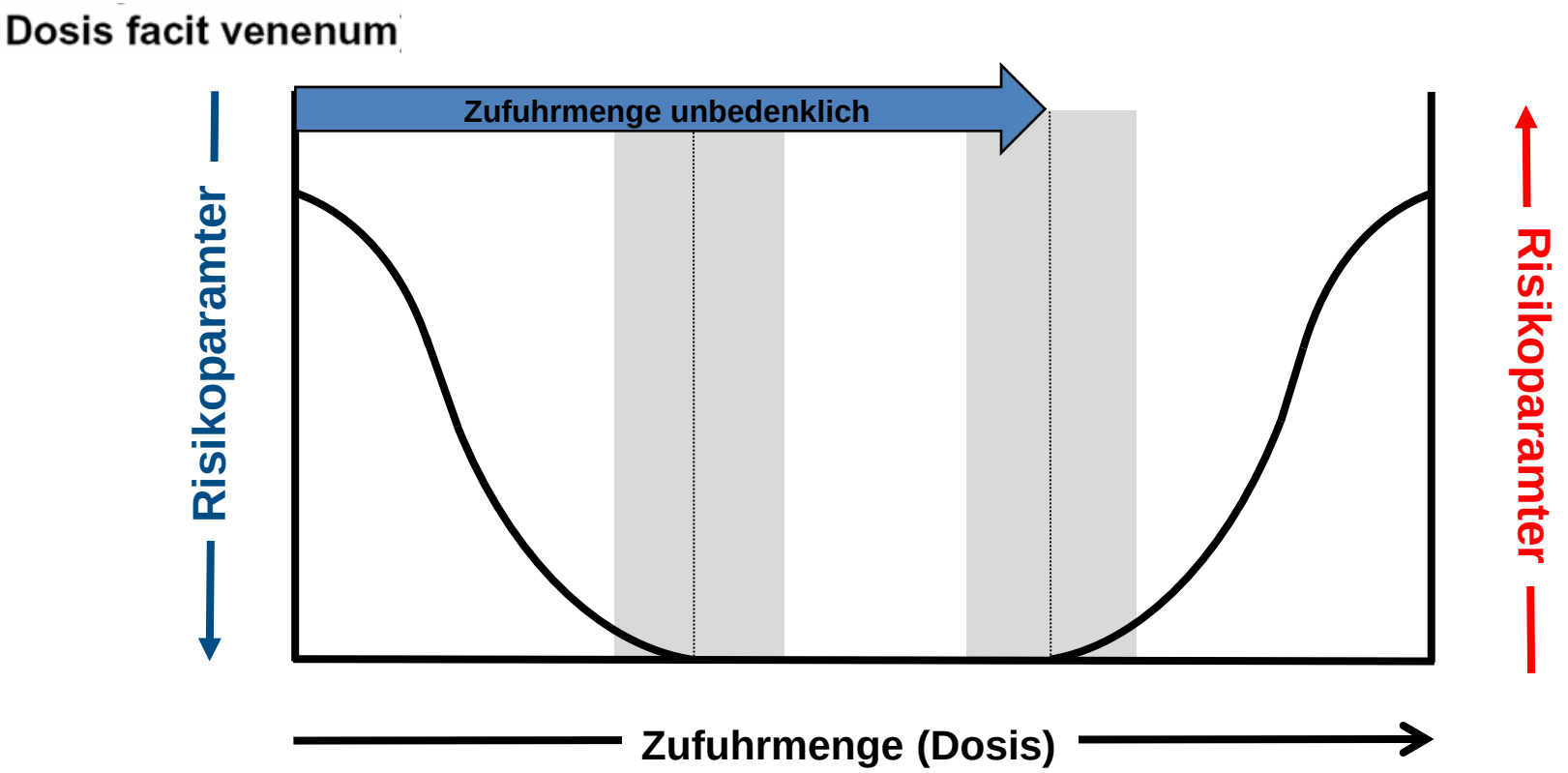






Biochemical Pharmacology 69 (2005) 395–406

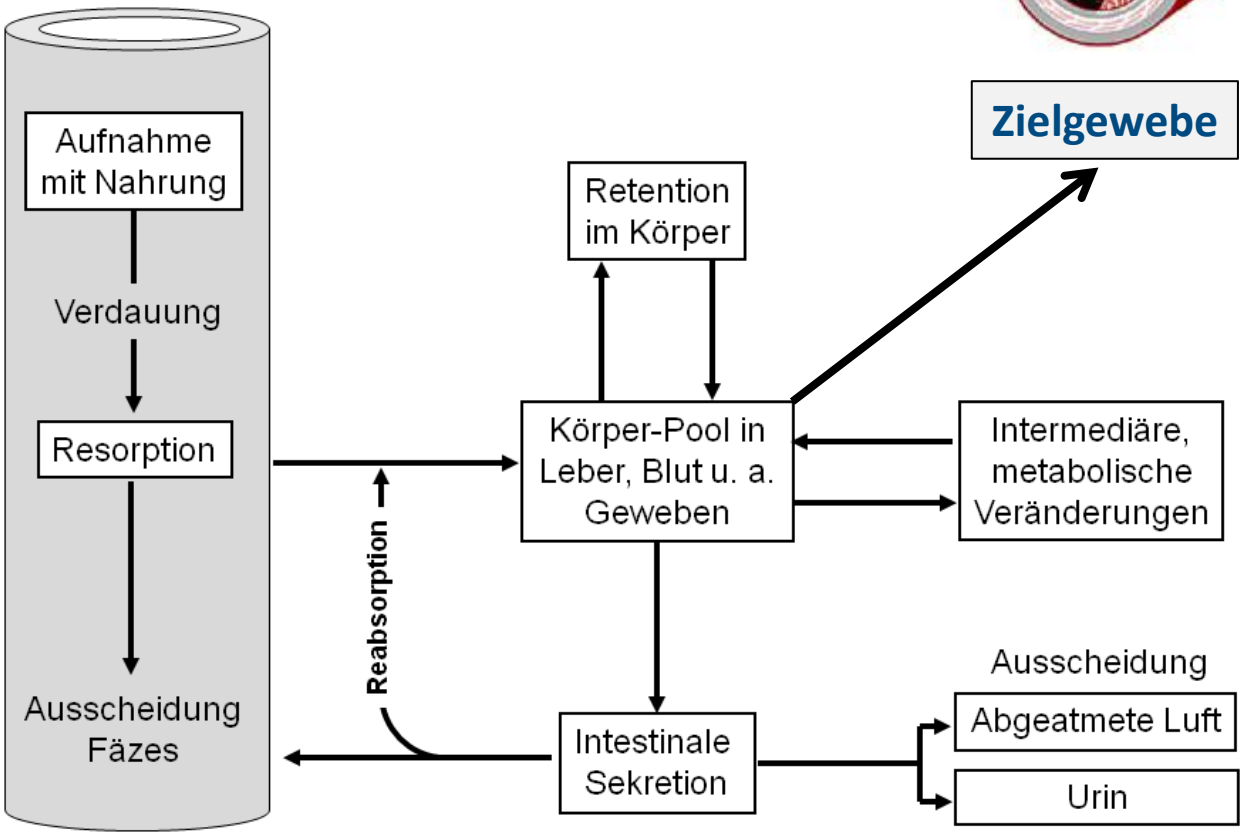
# *Totality of scientific evidence*

## Von der Plausibilität zur Dosis-Wirkungsbeziehung



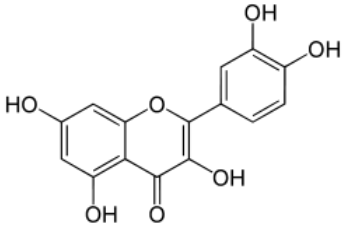
# *Totality of scientific evidence*

**A**bsorption
**D**istribution
**M**etabolismus
**E**xkretion

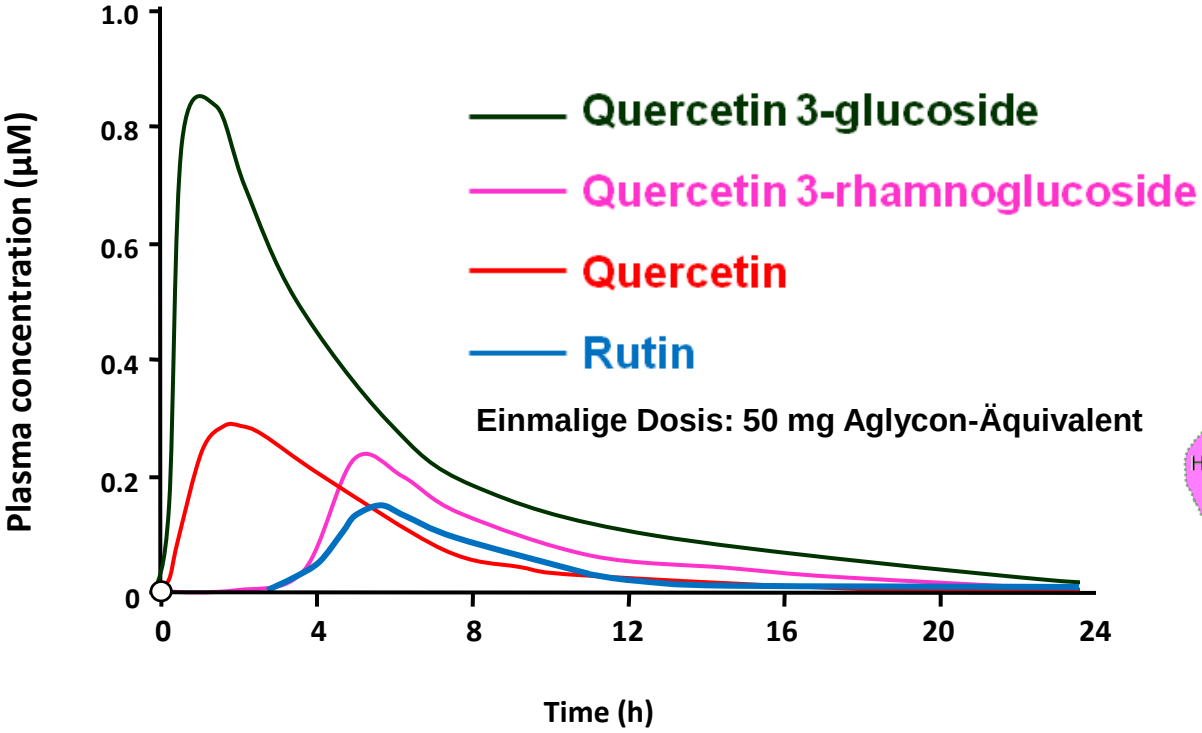



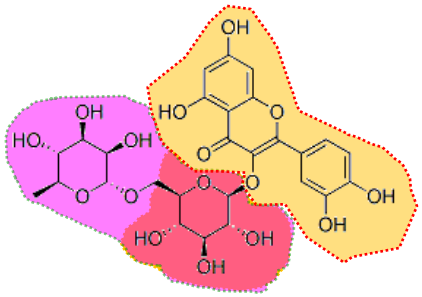
# Von der Zellkultur zum Menschen: ADME Probleme

# *Totality of scientific evidence*



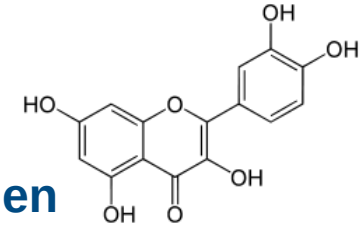

## Vergleich des Resorptionsverhaltens ausgewählter Quercetine



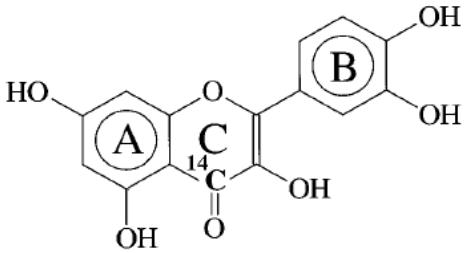



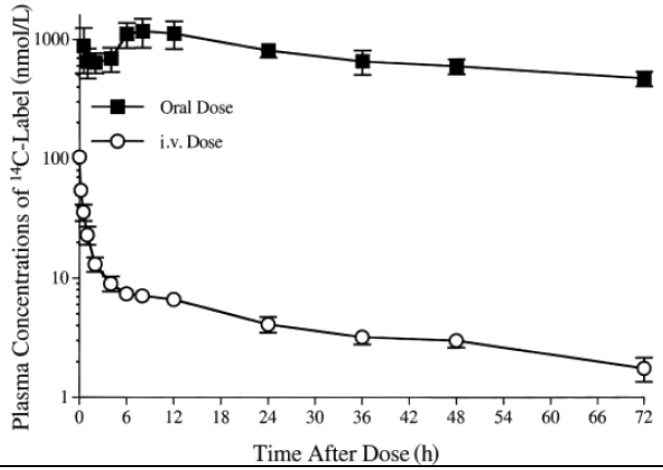

*Courtesy of Claudine Manach,* CHRU, *Clermont-Ferrand*

# *Totality of scientific evidence*




## Beispiel: Bioverfügbarkeit von Quercetin beim Menschen




**In a comparison of the two routes of administration it could be established that the oral absorption was as high as 36–54% among the six subjects studied, however, the bioavailability was close to zero.**

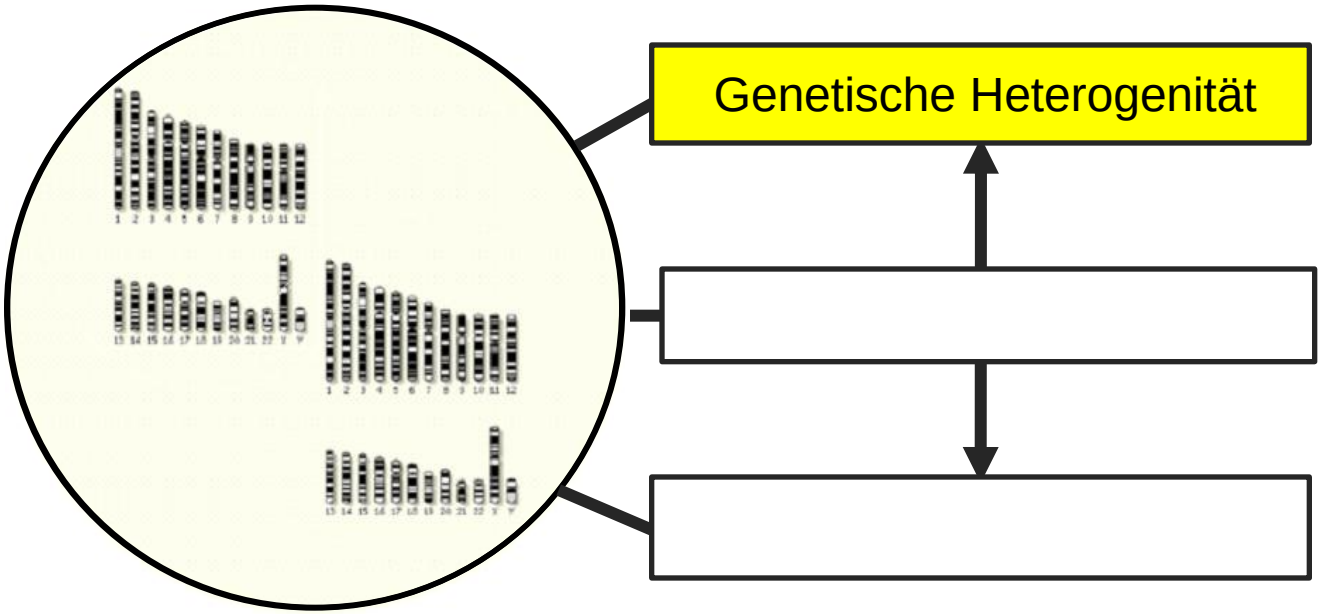
J. Nutr. 131 (2001), pp. 2648–2652

# Demonstration der Funktionalität in Humanstudien

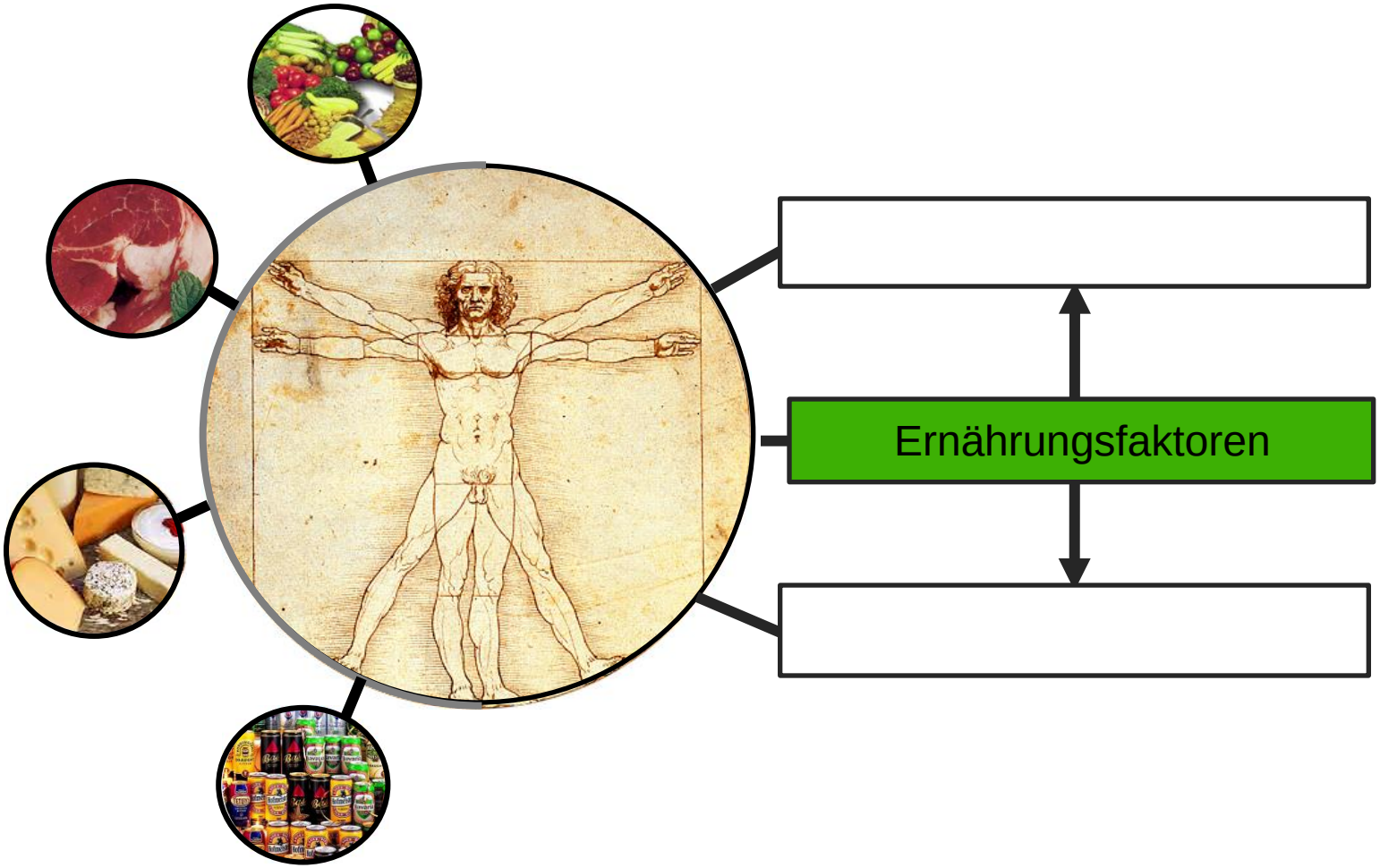
## Was bestimmt die Varianz im Menschen ?

# Determinanten einer funktionellen Wirkung von Lebensmitteln/Inhaltsstoffen

## Was bestimmt die Varianz im Menschen ?



# Determinanten einer funktionellen Wirkung von Lebensmitteln/Inhaltsstoffen



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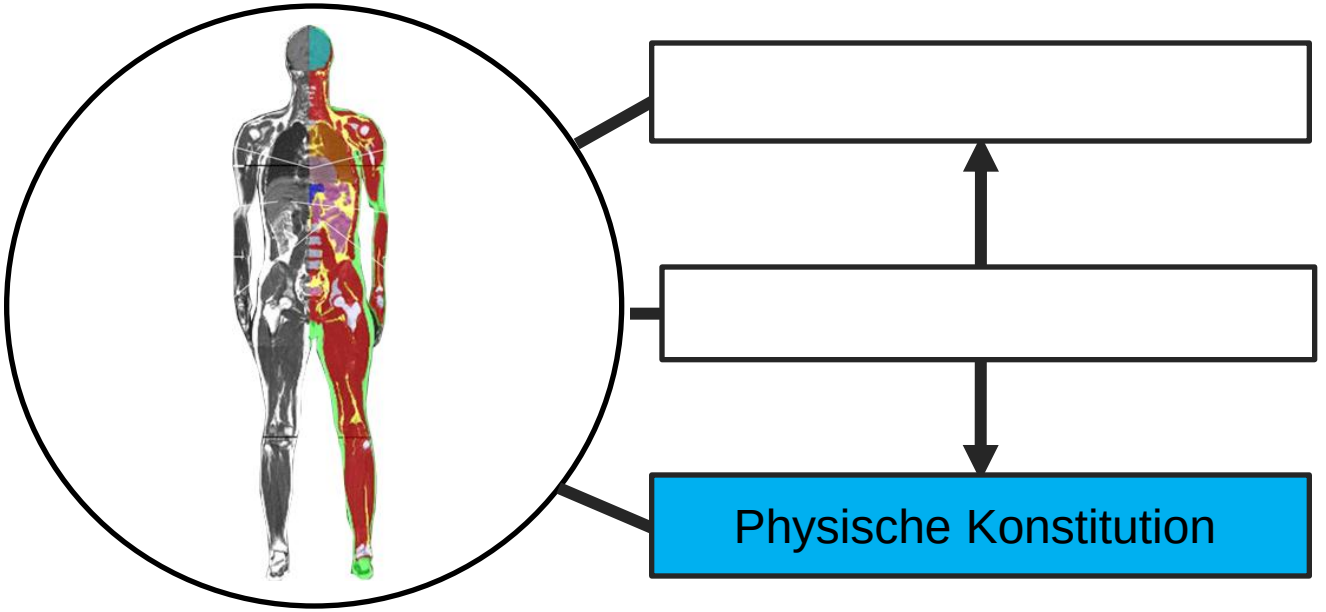

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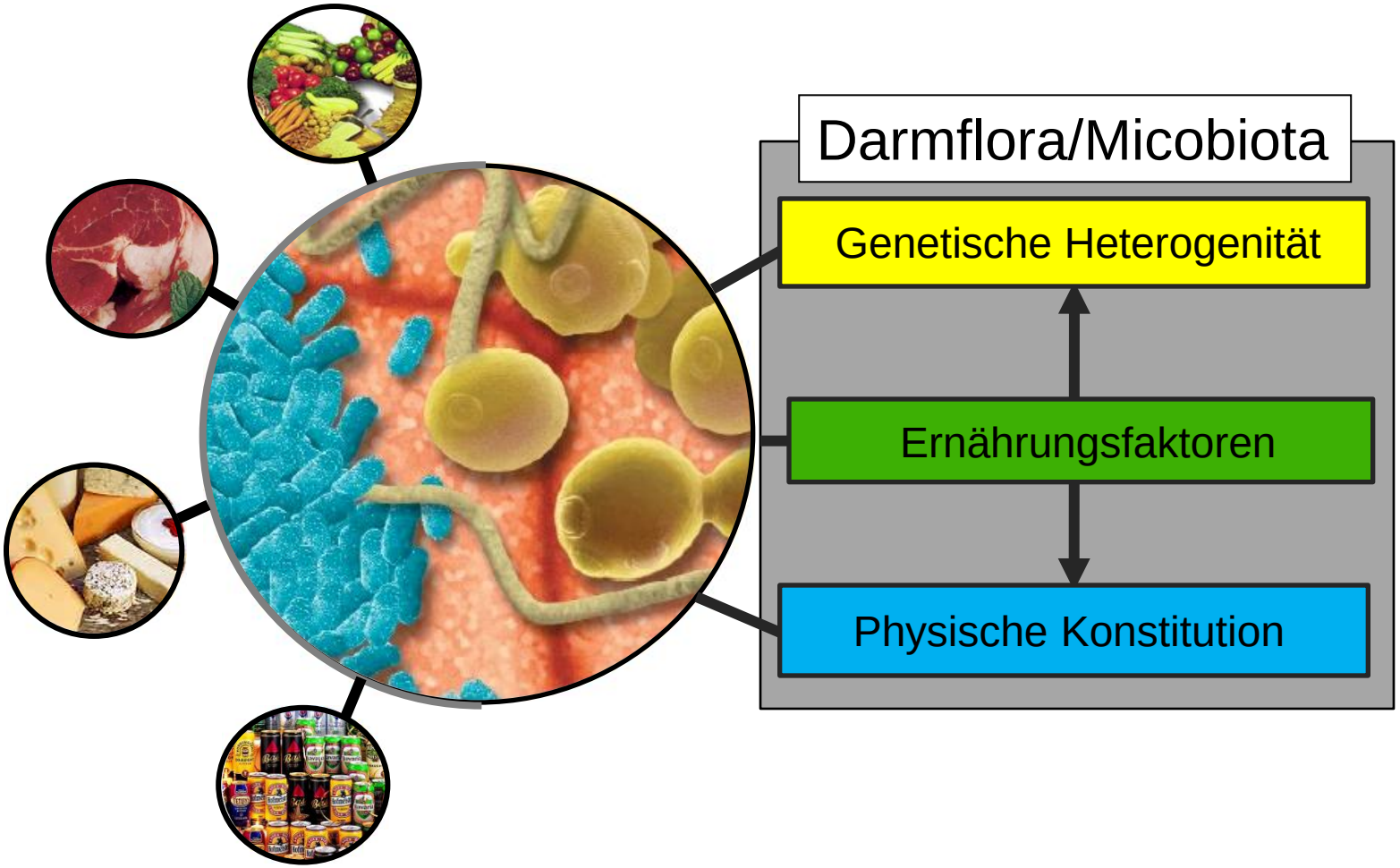
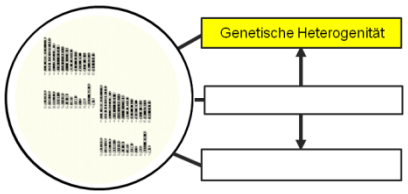
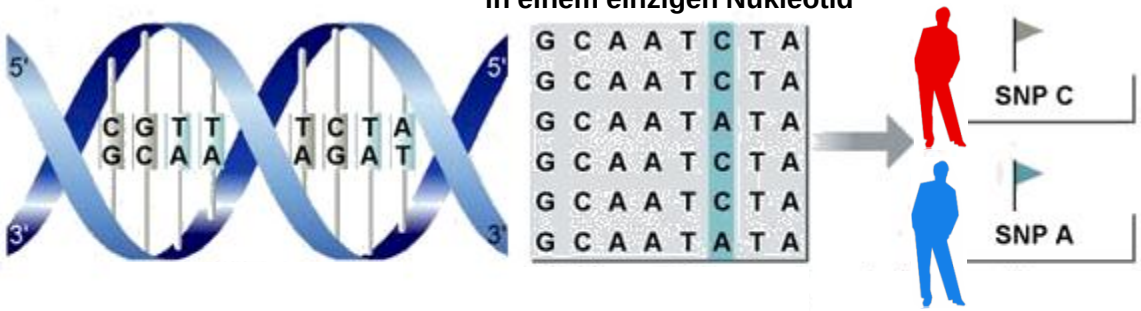


# Genetische Varianz als bedeutende Einflußgröße bei der individuellen metabolischen Antwort in Interventionen



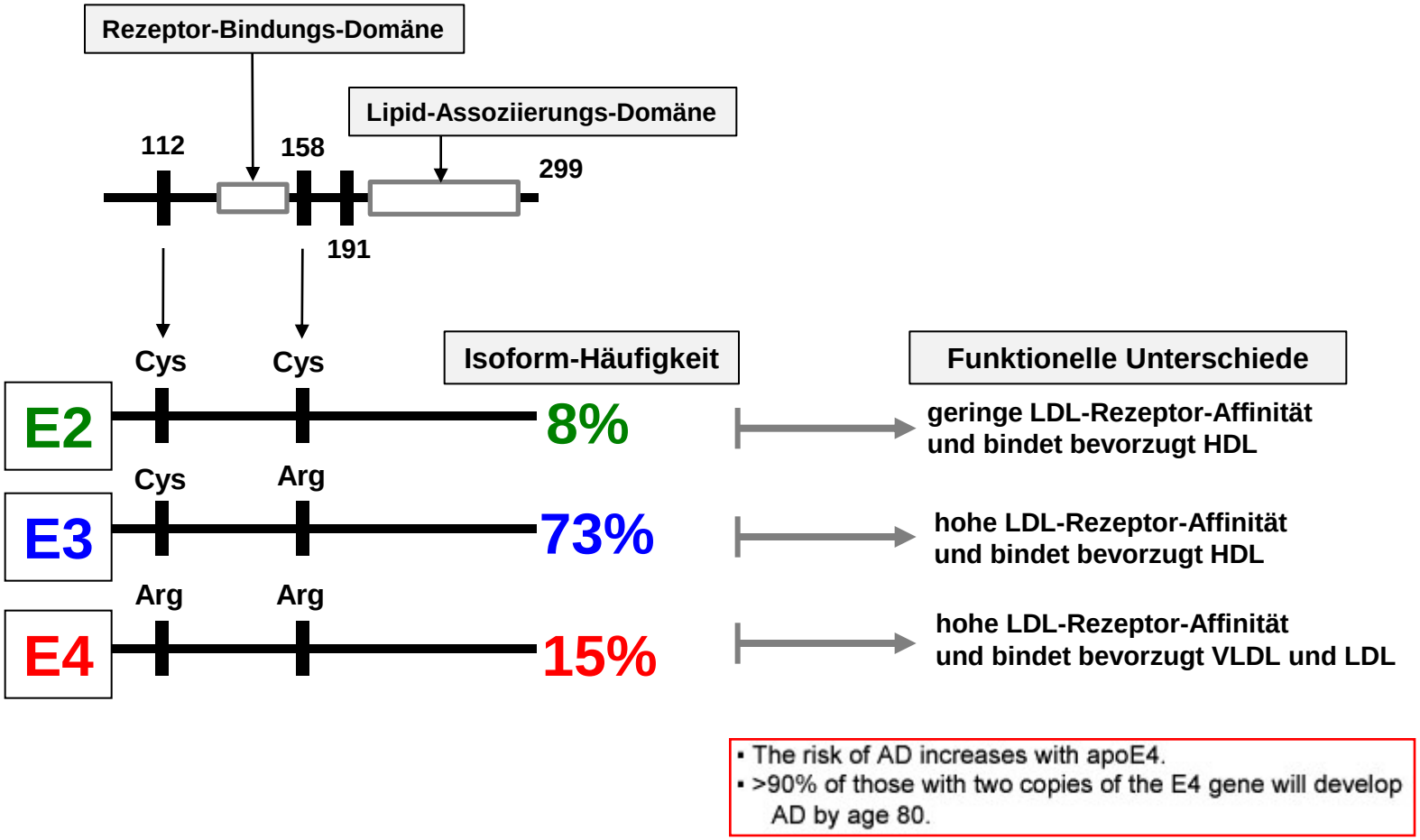

**Variation einer DNA-Sequenz in einem einzigen Nukleotid**




**Individuen mit unterschiedlichen SNP´s**

| | Unverändert | | | | SNP | | | |
|---|---|---|---|---|---|---|---|---|
| Gen: | AAG- | **C**GA- | ATT- | AGG | → AAG- | **G**GA- | ATT- | AGG |
| Protein: | Lys | **-Gln** | -Ile | -Arg | → Lys | **-Gly** | -Ile | -Arg |

# Genetische Varianz als bedeutende Einflußgröße bei der individuellen metabolischen Antwort in Interventionen



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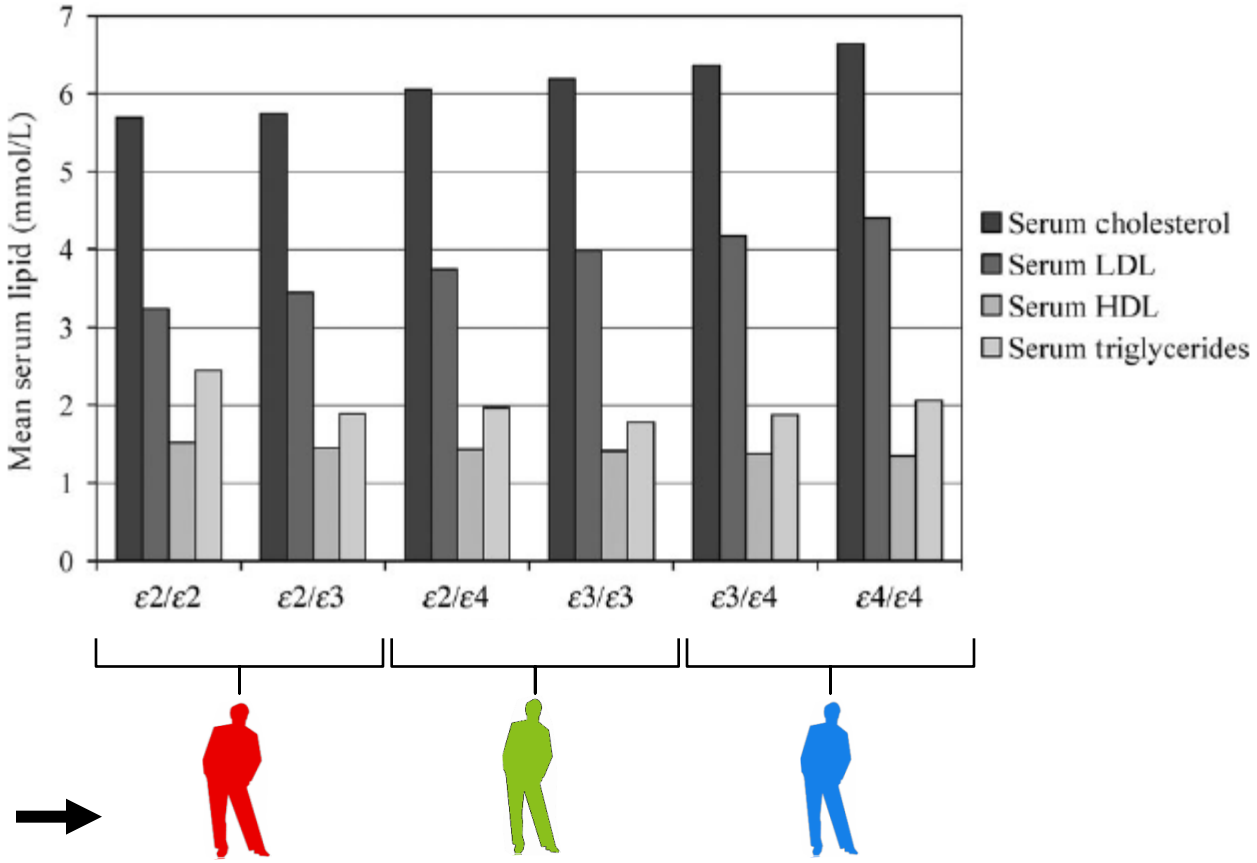



European Heart Journal (2007) **28**, 2930–2936

# Apo E Genotypen und die Heterogenität metabolischer Antworten auf diätetische Interventionen

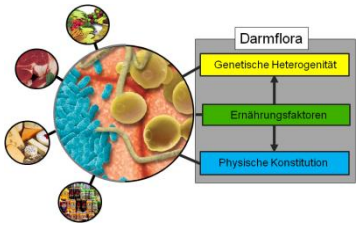
| | Apo E2 Response | | Apo E3 Response | | Apo E4 Response | |
|---|---|---|---|---|---|---|
| **Genotype** | 2/2 | 2/3 | 3/3 | 2/4 | 3/4 | 4/4 |
| ***Population Frequency*** | 1% | 10% | 62% | 2% | 20% | 5% |
| **Fish Oil[1]** | ↓↓TG<br>↓small dense LDL<br>↑HDL | | ↓TG<br>↓small dense LDL<br>↑HDL | | ↓TG<br>↓↓small dense LDL<br>↓HDL ↑↑LDL | |
| **Low Fat Diet[2,3]** | ↓LDL<br>↑small dense LDL | | ↓↓LDL<br>↔small dense LDL | | ↓↓↓LDL<br>↓small dense LDL | |
| **Moderate Fat Diet[3]** | ↔LDL<br>↔small dense LDL | | ↓LDL<br>↓small dense LDL | | ↓LDL<br>↑↑small dense LDL | |
| **Moderate Alcohol[4]** | ↑HDL ↓LDL | | ↑HDL | | ↓HDL ↑LDL | |
| **Effective Drug Response** | Atorvastatin<br>Pravastatin<br>Lovastatin | | No distinction | | Probucol<br>Simvastatin | |


Minihane AM *et al.* Arterioscler Thromb Vasc Biol (2000) 20:1990-1997.
Masson LF *et al.* Am J Clin Nutr (2003) 77:1098-111.
Moreno JA *et al.* J Nutr (2004) 134:2517-2522.
Corella D *et al.* Am J Clin Nutr (2001) 73:736-45
Marques-Vidal P *et al.* Obes Res (2003) 11:1200-6.
Mukamal KJ *et al.* Atherosclerosis (2004) 173:79-87
Bleich S *et al.* J Neural Trans (2003) 110:401-11.
Lussier-Cacan S *et al.* Arterioscler Thromb Vasc Biol (2002) 1:22:824-31.

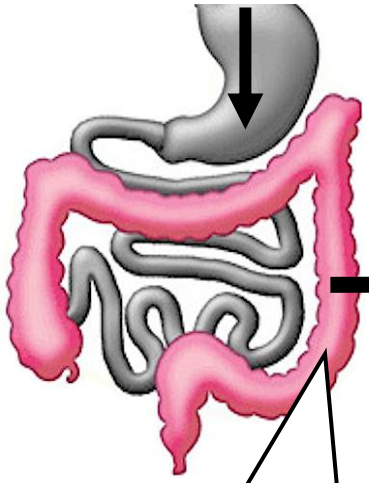


*British Journal of Nutrition* (2000), **83**, Suppl. 1, S127–S136

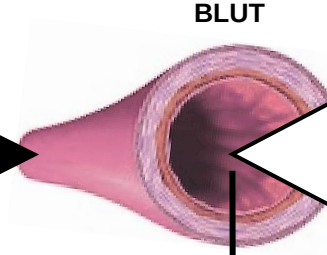
# Die Bedeutung der Darmflora in der Funktionalität von Nahrungsinhaltsstoffen

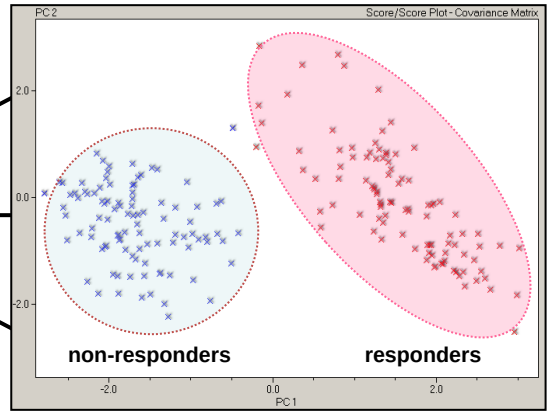



***Funktionelle Inhaltsstoffe***

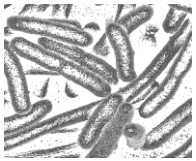


**BLUT**





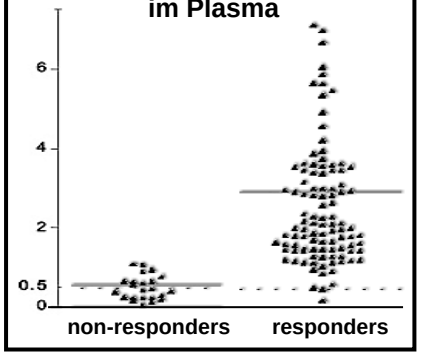

**Metabolische Umwandlung In der Darmflora**



Matairesinol
Secoisolariciresinol → Enterolacton

Daidzein → Equol

**Analyse der Metabolite im Plasma**



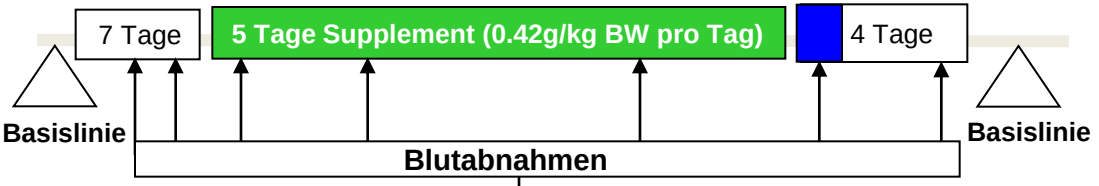
# *Beispiele aus der eigenen Forschung*

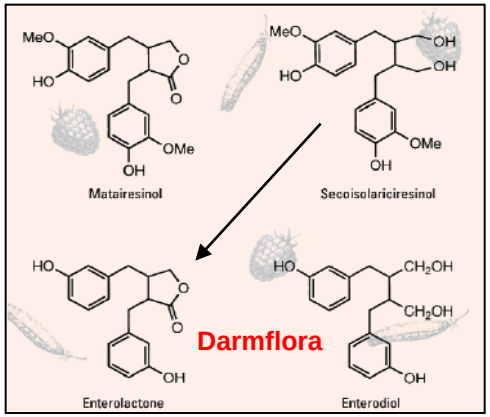
- **Biomarker-Identifizierung**

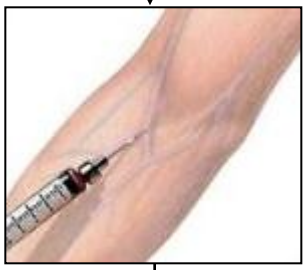
  - ***Leinsamen*** **Interventionsstudie**
  - ***ISOHEART*** **Interventionsstudie**

# Identifizierung von Biomarkern einer Leinsamen-Intervention

Gesunde Probanen (junge Männer)
erhielten 5 Tage jeweils morgens 0.4 g pro kg
Körpergewicht ein Leinsamen-Präparat

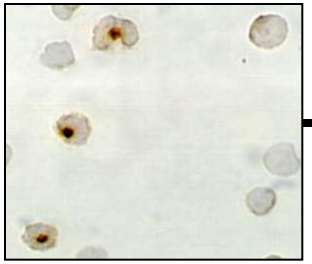


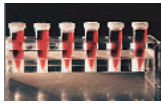
Blutabnahme

isolation der PBMC



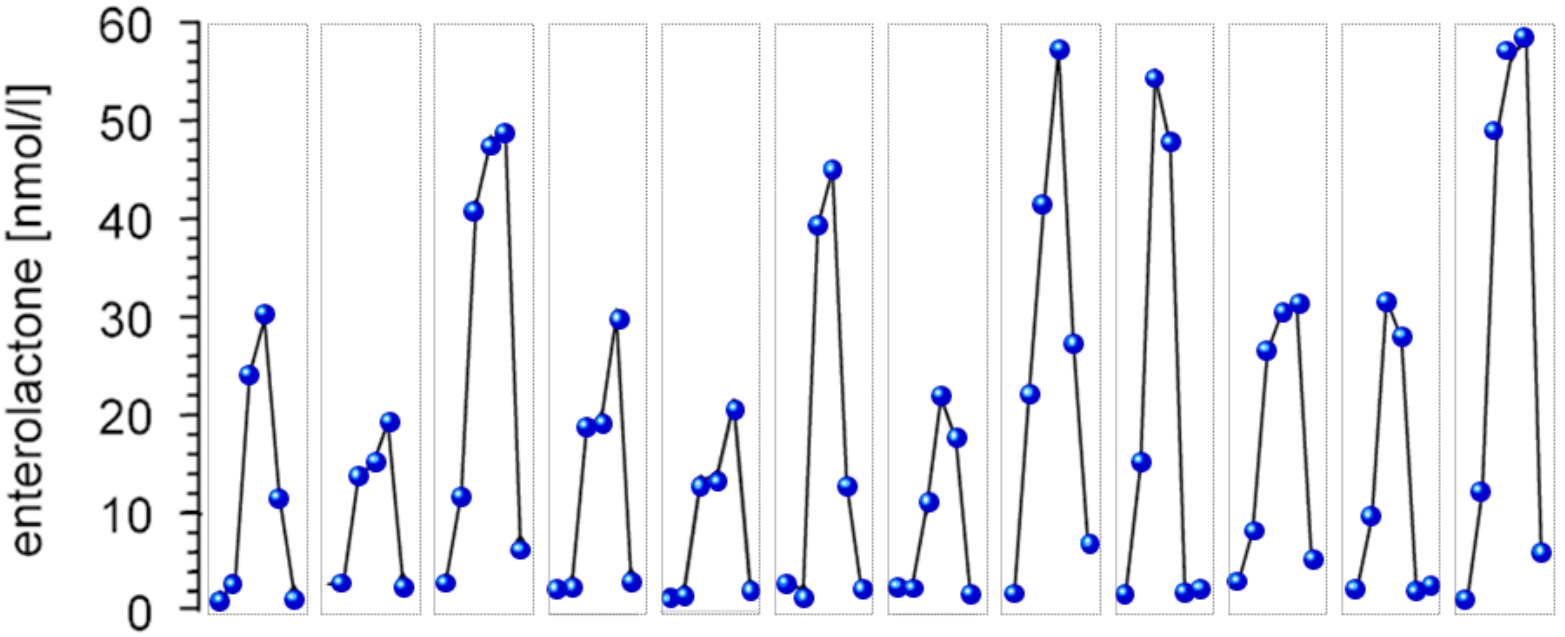
Transcriptom-Analyse

Proteom-Analyse



# Identifizierung von Biomarkern einer Leinsamen-Intervention

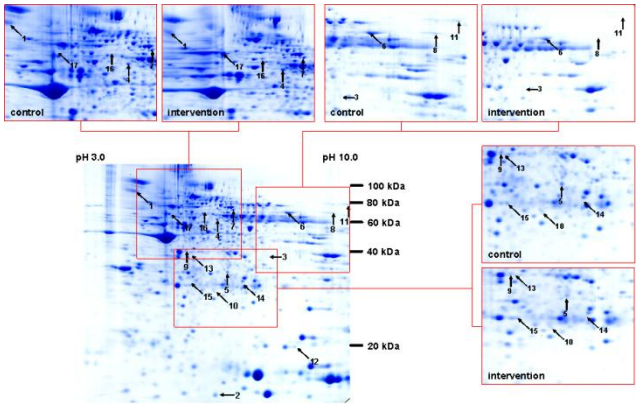
**Verlauf der Blutspiegel von Enterolacton der Probanden vor, während und nach der Leinsamen-Intervention**




| Phase | Day | Mean (nmol/L) | SEM | Median (nmol/L) | 25. Percentile | 75. Percentile |
|---|---|---|---|---|---|---|
| Pre-phase | -7 | 4.8 | 1.5 | 5.5 | 1.4 | 7.2 |
| | 0 | 8.4 | 3.9 | 5.4 | 1.4 | 14.2 |
| Intervention-phase | 2 | 24.9 | 5.9 | 21.8 | 8.1 | 42.4 |
| | 3 | 30.4 | 7.0 | 25.7 | 14.7 | 48.5 |
| | 7 | 22.0 | 5.2 | 14.8 | 12.1 | 32.0 |
| Washout-phase | 21 | 6.2 | 2.0 | 5.3 | 1.5 | 10.6 |

Proteomics. 2007 Sep;7(18):3278-88.

# Identifizierung von Biomarkern einer Leinsamen-Intervention

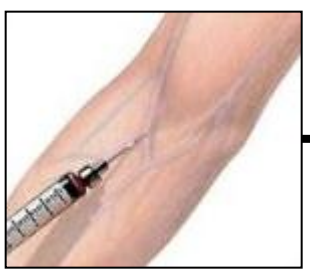


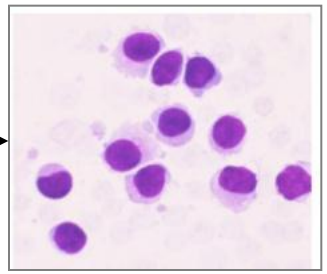

## Identifizierte Protein-Marker in PBMC

| Spot No | Protein description | Theoretical $M_r$/pI | Measured $M_r$/pI | Protein amount | | % Sequence-coverage | Accession No |
|---|---|---|---|---|---|---|---|
| | | | | Intervention phase/pre-phase | Washout-phase/pre-phase | | |
| | **Chaperons** | | | | | | |
| 1 | Chaperonin-containing TCP-1 beta subunit homolog | 96/6.4 | 58/6.0 | 2.05 | 0.86 | 36 | AAC98906 |
| 2 | Peroxiredoxin 4 | 38/5.3 | 31/5.9 | 3.70 | 1.05 | 64 | Q13162 |
| 3 | T-complex protein 1 subunit alpha (TCP-1-alpha) | 121/5.8 | 61/5.8 | 2.01 | 0.95 | 30 | P17987 |
| 4 | 60 kDa heat shock protein, mitochondrial precursor (hsp60) | 210/4.6 | 61/5.7 | only in intervention | n.d. | 41 | P10809 |
| | **Cytoskeletal proteins** | | | | | | |
| 5 | LIM protein | 48/7.7 | 38/7.6 | 2.88 | 1.55 | 37 | JC2324 |
| 6 | Beta 5-tubulin | 51/5.4 | 51/5.4 | 0.50 | 0.77 | 49 | AAH20946 |
| | **Metabolism** | | | | | | |
| 7 | Pyruvate kinase isozymes M1/M2 | 134/8.7 | 58/8.0 | 2.04 | 1.84 | 51 | P14618 |
| 8 | Protein-L-isoaspartate (D-aspartate) O-methyltransferase(EC 2.1.1.77) splice form I | 38/6.6 | 25/6.8 | 0.24 | 0.60 | 49 | P22061 |
| 9 | Long-chain-fatty-acid beta-oxidation multienzyme complex alpha chain precursor, mitochondrial | 160/10.4 | 160/10.4 | only in pre-phase | 0.99 | 35 | P40939 |
| 10 | Cyclophilin A | 21/8.7 | 18/7.7 | only in pre-phase | 0.76 | 50 | P62937 |
| 11 | TALDO 1 protein | 54/5.4 | 37/5.8 | only in intervention | only in intervention | 26 | AAH18847 |
| 12 | Phosphoglycerate mutase 1 (Phosphoglycerate mutase isozyme B) (PGAM-B) (BPG-dependent PGAM 1) | 39/6.9 | 29/6.8 | 2.86 | 1.65 | 56 | P36871 |
| | **Gene regulation** | | | | | | |
| 13 | Purine-nucleoside phosphorylase (EC 2.4.2.1) | 47/6.9 | 32/6.5 | 2.06 | 1.89 | 62 | P00491 |
| 14 | Stress-induced-phosphoprotein 1 | 154/6.8 | 63/6.4 | 2.01 | only in pre-phase | 44 | P31948 |
| | **Other proteins** | | | | | | |
| 15 | Platelet glycoprotein IIIa/II | 220/4.5 | 86/5.0 | 0.45 | 0.52 | 23 | B36268 |
| 16 | Chain B, Crystal Structure Of Desoxy-Human Hemoglobin Beta6 Glu->trp | 13/6.5 | 16/7.3 | 0.30 | 0.54 | 67 | 6HBWB |
| 17 | Gelsolin precursor | 103/9.6 | 86/5.9 | only in pre-phase | 1.42 | 23 | P06396 |

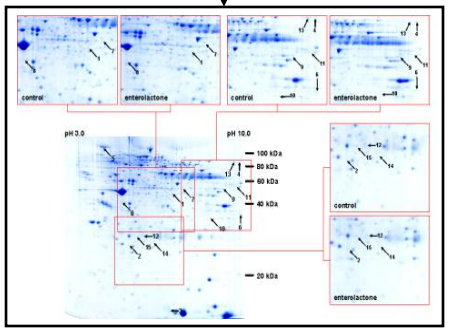
Proteomics. 2007 Sep;7(18):3278-88.

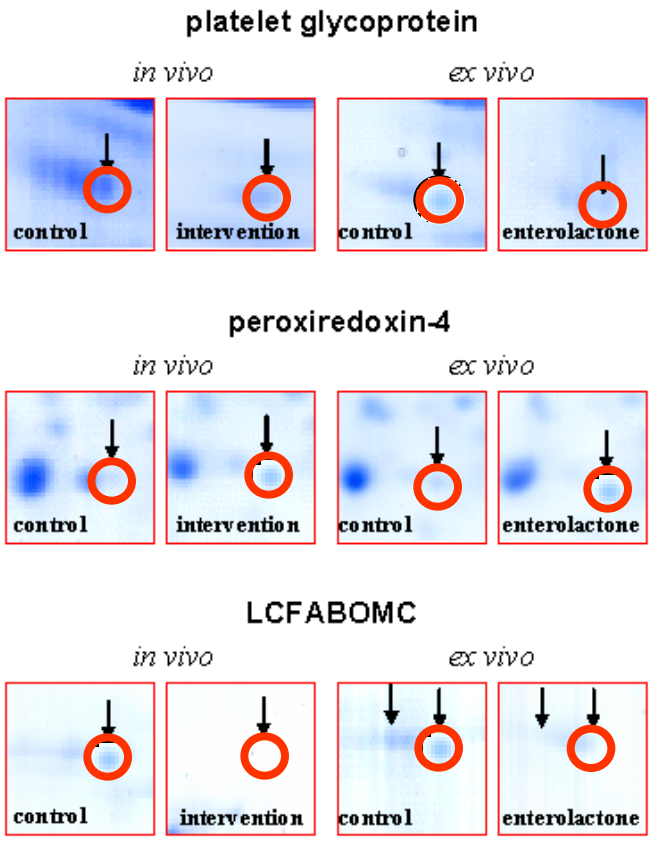
# Identifizierung von Biomarkern einer Leinsamen-Intervention

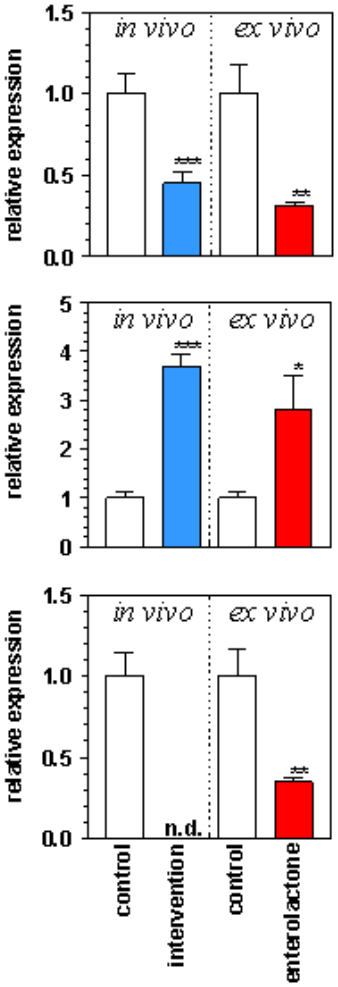




PBMC exponiert gegenüber
50 nM Enterolacton ex vivo





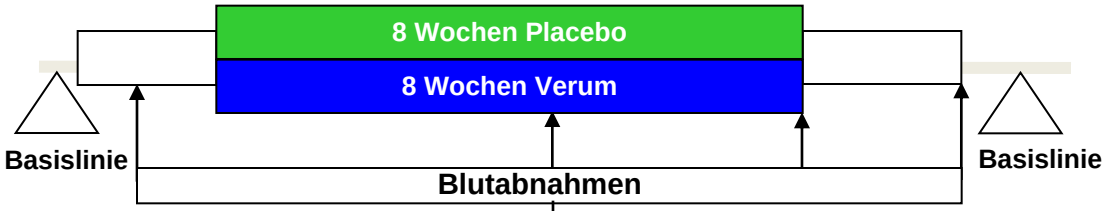




Proteomics. 2007 Sep;7(18):3278-88.

# Identifizierung von Biomarkern einer Soja-Intervention

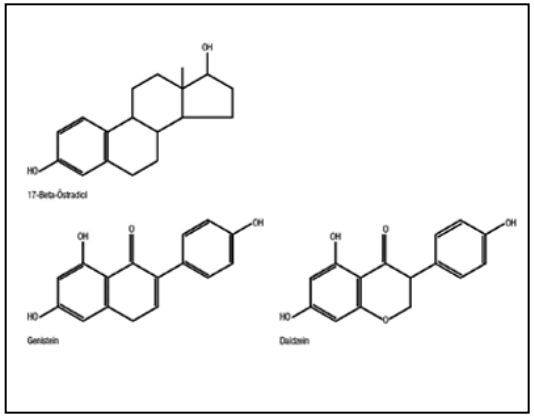
120 postmenopausale Frauen

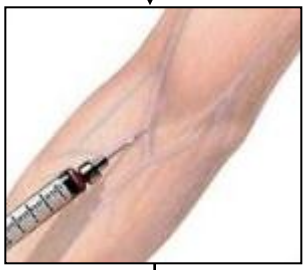
Insgesamt 120 postmenopausale Frauen
2 Riegel pro Tag mit jeweils 25 mg Genistein/Daidzein (2:1)




120 postmenopausale Frauen

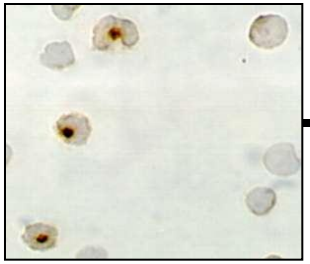
→ 20 Probanden in Verum
20 Probanden in Placebo



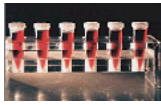
Blutabnahme

isolation der PBMC

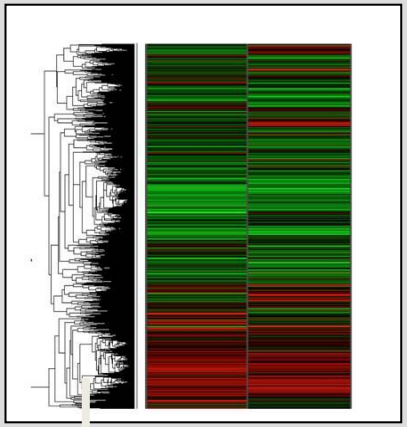


Transcriptom-Analyse

Proteom-Analyse

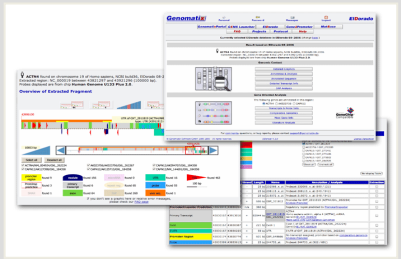


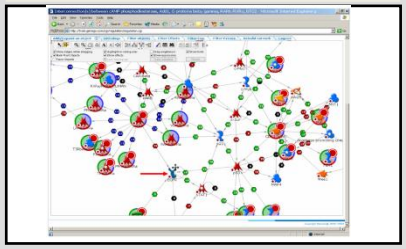
# Identifizierung von Biomarkern einer Soja-Intervention

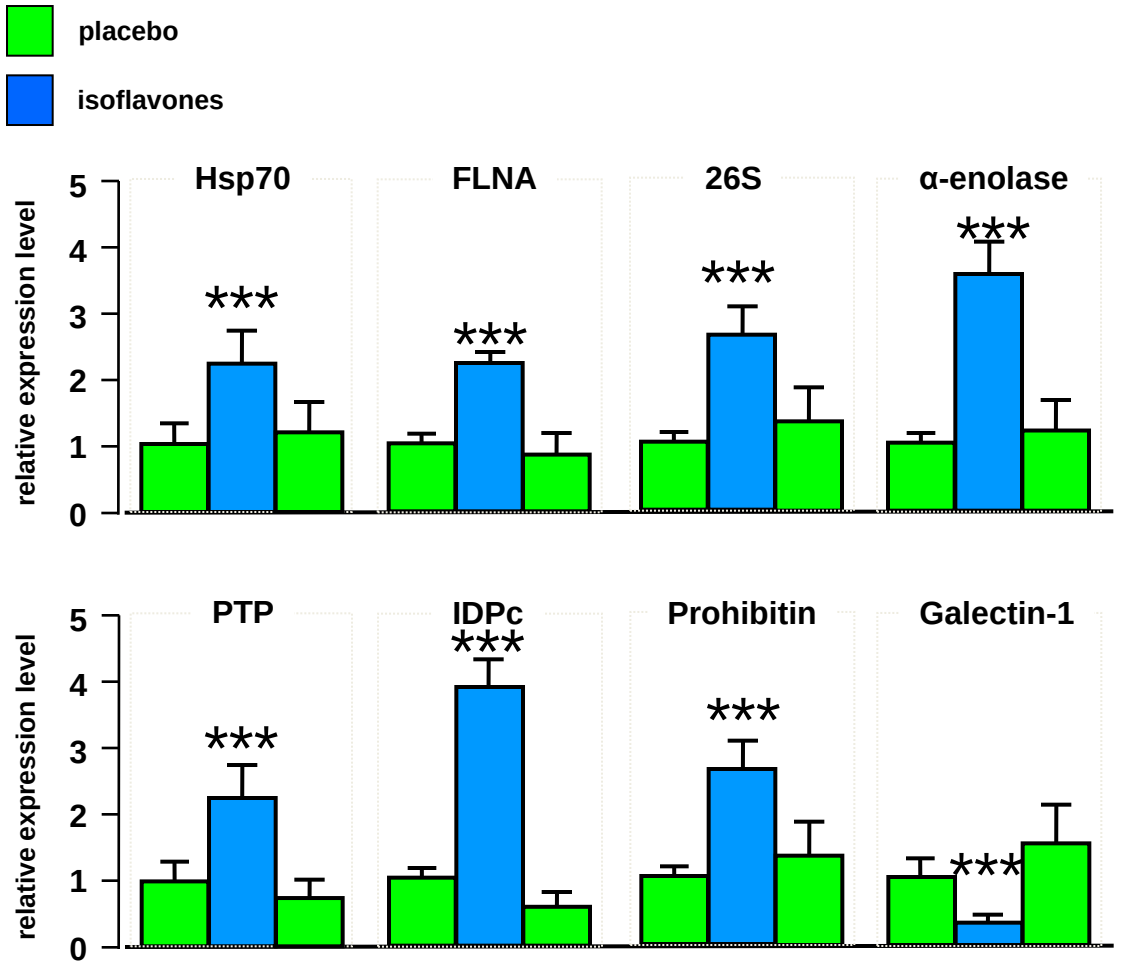


Promotor analysis and prediction

Pathway analysis tools








Am J Clin Nutr, Vol. 86, No. 5, 1369-1375, November 2007

# Identifizierung von Biomarkern einer Soja-Intervention

## Clinical (inflammatory markers) of the volunteers undergoing isoflavone treatment

Plasma inflammatory factor concentrations at baseline (*t*0) and week 8 (*t*8) of the isoflavone and placebo intervention arms[1]

| | Isoflavones | | Placebo | | |
|---|---|---|---|---|---|
| | *t*0 | *t*8 | *t*0 | *t*8 | $P^2$ |
| vWF (IU/dL) | 104.96 ± 53.77 [116] | 105.46 ± 53.07 [116] | 103.27 ± 49.46 [115] | 99.99 ± 39.92 [116] | 0.883 |
| sICAM-1 (ng/mL) | 215.04 ± 51.60 [116] | 220.40 ± 52.77 [117] | 217.45 ± 52.21 [116] | 217.78 ± 48.28 [115] | 0.147 |
| sVCAM-1 (ng/mL) | 504.79 ± 134.39 [114] | 503.48 ± 146.66 [113] | 498.14 ± 129.00 [114] | 499.76 ± 135.88 [111] | 0.475 |
| E-selectin (ng/mL) | 42.14 ± 15.41 [117] | 42.17 ± 15.82 [117] | 40.67 ± 15.05 [117] | 41.26 ± 15.17 [117] | 0.307 |
| MCP-1 (ng/mL) | 259.36 ± 95.93 [117] | 260.43 ± 101.23 [117] | 262.40 ± 85.74 [117] | 260.49 ± 106.17 [117] | 0.928 |
| Endothelin-1 (pg/mL) | 1.15 ± 0.39 [107] | 1.20 ± 0.43 [107] | 1.15 ± 0.39 [106] | 1.21 ± 0.40 [107] | 0.800 |
| hs-CRP (mg/L)[3] | 1.71 ± 1.89 [114] | 1.70 ± 1.89 [113] | 1.64 ± 1.73 [116] | 1.76 ± 1.83 [113] | 0.086 |

[1] All values are $\bar{x}$ ± SD, *n* in brackets. vWF, von Willebrand Factor; sICAM-1, soluble intracellular adhesion molecule 1; sVCAM-1, soluble vascular cell adhesion molecule 1; MCP-1, monocyte chemoattractant protein 1; hs-CRP, highly sensitive C-reactive protein.

Urinary isoflavone yields at baseline (*t*0) and week 8 (*t*8) of the isoflavone and placebo intervention arms[1]

| | Isoflavones | | Placebo | | |
|---|---|---|---|---|---|
| | *t*0 | *t*8 | *t*0 | *t*8 | $P^2$ |
| Genistein (mg/d) | 0.37 ± 0.40 [114] | 7.27 ± 3.58 [117] | 0.37 ± 0.40 [117] | 0.42 ± 0.32 [117] | < 0.0001 |
| Daidzein (mg/d) | 0.16 ± 0.22 [114] | 5.76 ± 2.70 [117] | 0.22 ± 0.30 [117] | 0.22 ± 0.32 [117] | < 0.0001 |
| Equol (mg/d) | 0.08 ± 0.06 [114] | 0.85 ± 1.43 [117] | 0.08 ± 0.05 [117] | 0.11 ± 0.08 [117] | < 0.0001 |
| Equol producers[3] | 0.10 ± 0.05 [31] | 2.61 ± 1.73 [33] | 0.09 ± 0.05 [33] | 0.13 ± 0.09 [33] | < 0.0001 |
| Equol nonproducers[4] | 0.07 ± 0.06 [82] | 0.15 ± 0.08 [83] | 0.08 ± 0.05 [84] | 0.094 ± 0.08 [83] | < 0.0001 |

[1] All values are $\bar{x}$ ± SD, *n* in brackets.

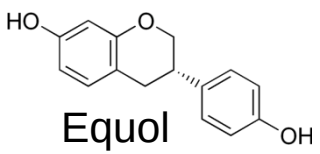
The relative binding affinity of the R- and S-equol enantiomers for ERa were 0.47% and 2.0% with that of 17ß-estradiol. However, S-equol is largely ERß selective and has a relatively high affinity for this receptor subtype. S-equol binds ERß with around 20% of the affinity of 17ß-estradiol (equol: $K_i$ = 0.7 nmol/L; 17ß-estradiol: $K_d$ = 0.15 nmol/L), whereas the R enantiomer only has 1% of the affinity.

Am J Clin Nutr. 2006 Mar;83(3):592-600.

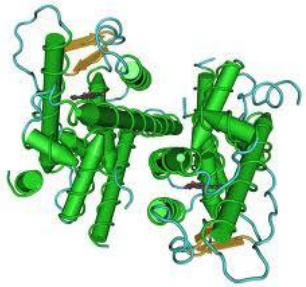
# Identifizierung von Biomarkern einer Soja-Intervention

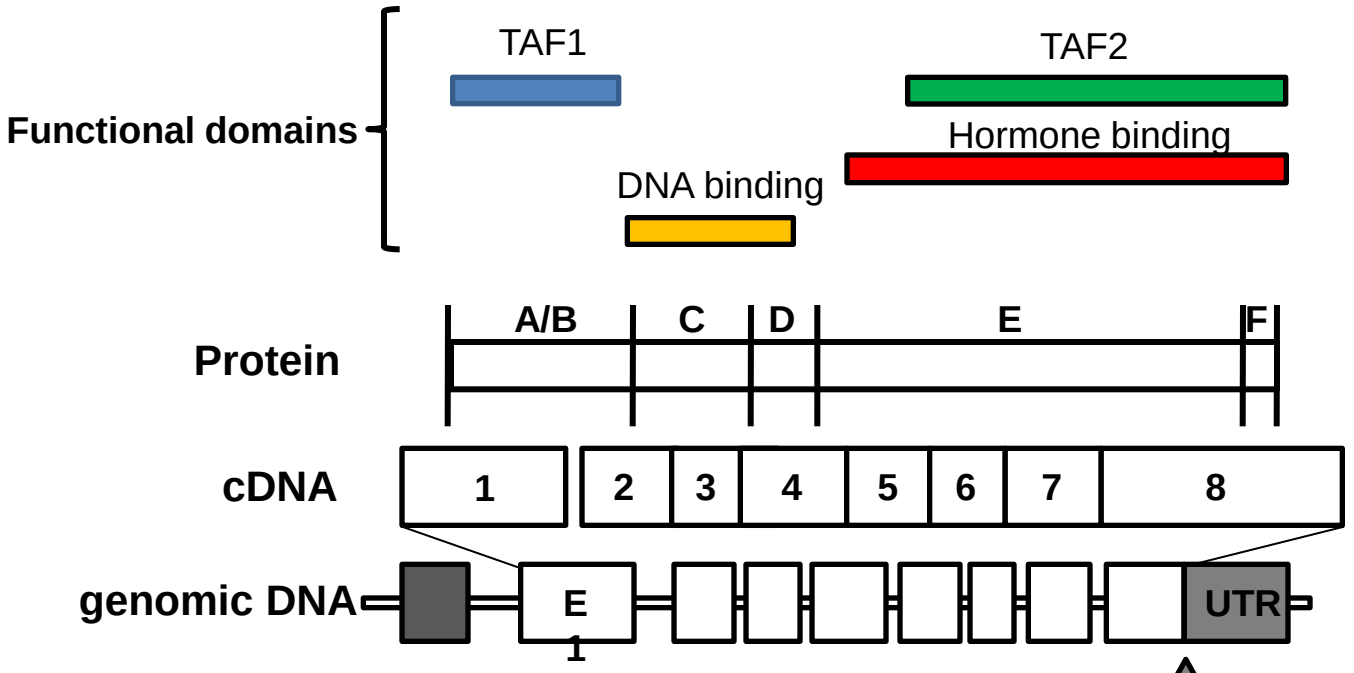
**Genetic variability as a determinant for the differences in the response to soy isoflanones**

## The estrogen receptor ß




Equol



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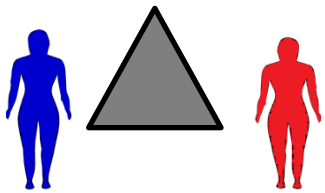
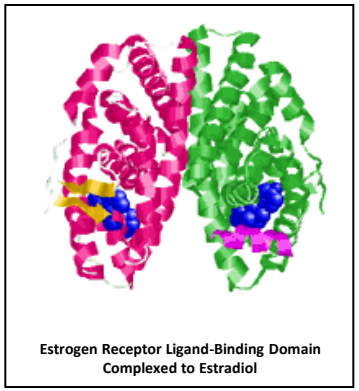
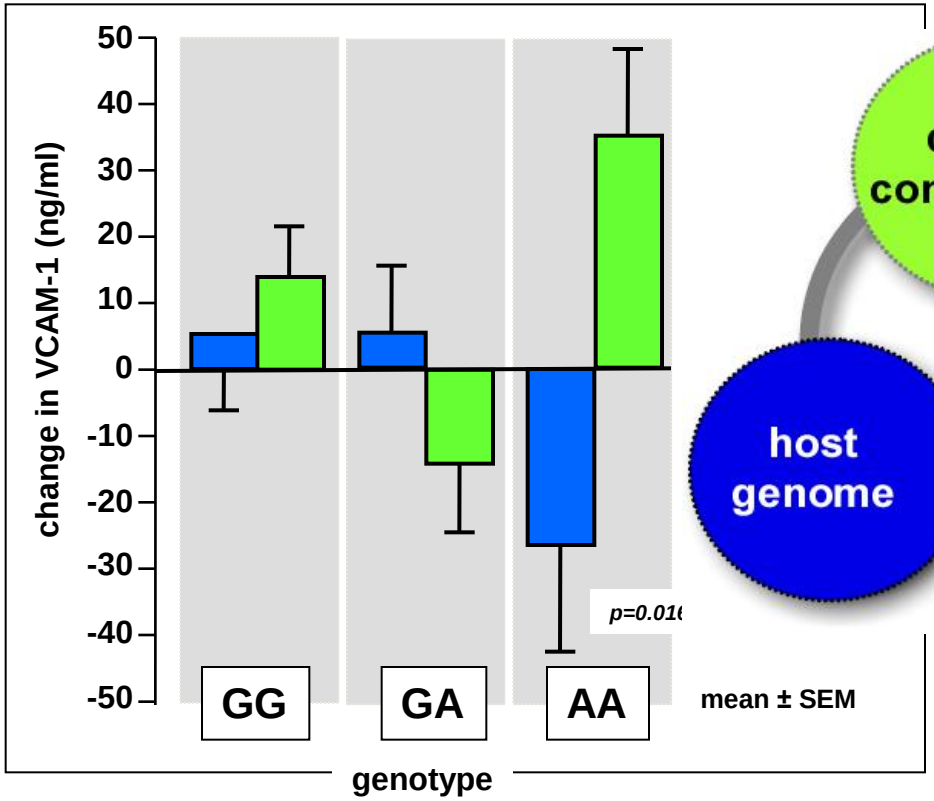
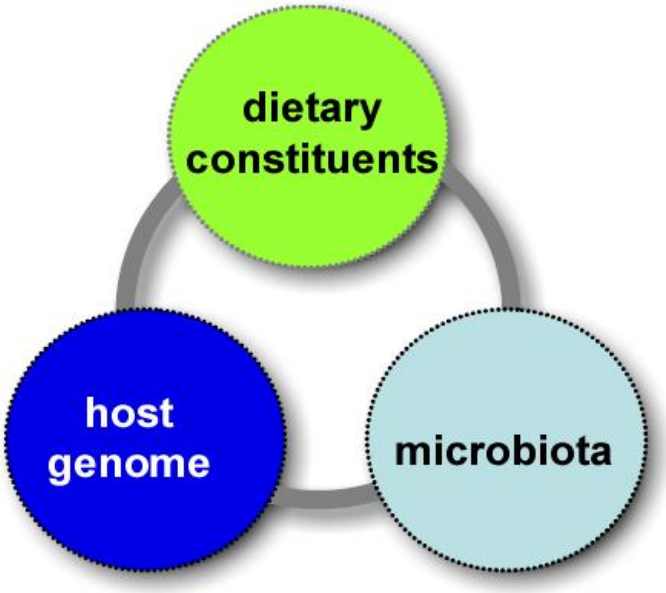


# Identifizierung von Biomarkern einer Soja-Intervention

**Plasma vascular cell adhesion molecule 1 (VACM-1) concentrations in the volunteers according to estrogen receptor ß*Alu*I genotype and treatment**




Am J Clin Nutr. 2006 Mar;83(3):592-600.

# Zusammenfassung

- „Ehrliche“ Produkte und tragfähige Claims gibt es nicht umsonst!
- Das sich nun „herausstellende Prinzip“ in der Vorgehensweise des NDA Panels ist „fair“ und folgt der Einsicht „was Wissenschaftler für Wissenschaft“ halten.
- Plausibilitäten und Analogien sind nur in begrenztem Umfang übertragbar – das gilt vor allem für „new chemicals“!
- Zwei Humanstudien (in der Zielgruppe) sind/sollten „*minimal* standard“ sein“!

***ABER .....***

# Zusammenfassung

- **Health claims erfordern eine hohen Aufwand (im Antragsverfahren) und für den Nachweis der Sicherheit und Wirksamkeit.**
- **Investitionen sind mit Blick auf das „return of investment“ schwer kalkulierbar.**
- **Es resultiert eine „Monopolisierung“; d.h., kleine und mittelständische Unternehmen sind von der Entwicklung fast ausgegrenzt (sowohl wegen know-how als auch über den finanziellen R&D Aufwand).**
- **Die Entwicklung hat nun aber auch Auswirkungen auf die akademische Forschung im Feld, da dass Interesse der Unternehmen sinkt und weniger PPP eingegangen werden.**