Metabolic deregulations in metabolic diseases and cancer

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Outline

1. Substrate preference and metabolic flexibility
2. Measuring metabolic flexibility in vivo and in vitro
3. Implications of disturbances in metabolic flexibility
4. Associations between metabolic diseases and cancer
5. Prevention of metabolic diseases and cancer
Metabolism is an integrated process
At the whole body level

Fuel exchange during fasting

Glucoseuptake
Glycogen
Glycogen
Glycogen
Glycogen
Glycogen
Fuel exchange after a meal

- Glucose uptake
- Glycogenogenesis
- Neoglucogenesis

β-cells

↑ Glucose uptake
↑ Glycogenogenesis
↓ Neoglucogenesis

Progression to type 2 diabetes
Oral glucose tolerance test

Blood glucose (g/L)

Healthy
Prediabetes
T2D

Blood insulin (μU/mL)
Metabolism is an integrated process
At the cellular level

KEGG - Metabolic pathways - Reference pathway

Nutrient uptake and metabolism

KEGG - Metabolic pathways - Reference pathway

https://www.diapedia.org/metabolism-and-hormones/5105765817/metabolic-pathways
Glucose metabolism

Glycolytic intermediates provide the substrates for the pentose phosphate pathway (PPP) that generates the ribose 5-phosphate that is critical for nucleotide biosynthesis. The oxidative arm of the PPP, which utilizes glucose 6-phosphate, additionally generates NADPH. Glycerol 3-phosphate, which contributes the glycerol head groups for phospholipid biosynthesis, is formed from the intermediate dihydroxyacetone phosphate. 3-Phosphoglycerate provides the foundation for the serine synthesis pathway, which can further fuel glycine production for protein synthesis. This pathway also contributes to the pool of one-carbon units (CH2-THF) that are used in nucleotide biosynthesis. Glucose-derived citrate provides the acetyl-CoA that represents the fundamental building block for the synthesis of the fatty acids that comprise cellular lipids. Key metabolic enzymes are shown in blue; NADPH required for biosynthetic reactions is shown in green. CH2-THF, methylene tetrahydrofolate; GLDC, glycine decarboxylase; PHGDH, phosphoglycerate dehydrogenase; PKM2, pyruvate kinase M2 isoform; PPP, pentose phosphate pathway; THF, tetrahydrofolate.

Lipid metabolism

AMPK activation

- increased uptake of glucose (↑ GLUT4)
- increased oxidation of glucose (↑ HKII)
- Inhibition of glycogen synthesis (↓ GS)
- increased uptake of fatty acids (↑ FAT/CD36)
- Increased fatty acid oxidation (↓ ACC)

Metabolic changes known to be induced by AMPK in muscle, including stimulation of glucose and fatty acid uptake, fatty acid oxidation, and mitochondrial biogenesis, and inhibition of glycogen synthesis and, via inhibition of TOR, hypertrophy. Question marks indicate that the direct target for AMPK responsible for the observed downstream effect is not known. The effect on fatty acid uptake has to date only been observed in cardiac muscle.

Modulation of substrate preference

Mechanism of reciprocal inhibition of glucose and fatty acid oxidation.

Mechanism of reciprocal inhibition of glucose and fatty acid oxidation. When glucose uptake and consumption increases, fatty acid oxidation is suppressed by malonyl-CoA’s allosteric inhibition of CPT-1, and increased pyruvate from glycolysis inhibits PDK, which stimulates glucose oxidation (yellow lines). CPT-1 inhibition increases the concentration of LCFA-CoA, which then are used for triglyceride synthesis and stored (pink arrow). Vice versa, when fatty acid oxidation is high, glucose uptake, glycolysis, and pyruvate oxidation are decreased (red lines) because rising levels of acetyl-CoA and NADH impede PDH activity. Additionally, increased citrate levels inhibit GLUT4 and PFK-1. PFK-1 inhibition results in increased glucose-6-phosphate concentrations that inhibit HK. A decrease in pyruvate oxidation enables pyruvate to be used as either a gluconeogenic precursor or, in energetically demanding tissues, a substrate for PC, which produces oxaloacetate that is used as anaplerotic substrate (purple arrows). During caloric restriction, the rise in AMP/ATP activates AMPK, which inhibits ACC, stimulating fatty acid uptake by the mitochondria via CPT-1. ACL, ATP-citrate lyase; CACT, carnitine acylcarnitine translocase; CTP, citrate transport protein; CYTO, cytosol; FAS, fatty acid synthase; LCFA, long-chain fatty acid; MITO, mitochondria; MPC, mitochondrial pyruvate carrier; PC, pyruvate carboxylase. Green arrows indicate stimulatory reactions.

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Metabolic flexibility
The capacity for an organism to adapt fuel oxidation to fuel availability

The ability to switch from one substrate to another is crucial to maintain whole body energy balance and a healthy storage of energy.

Methodology

MEASURING METABOLIC FLEXIBILITY IN VIVO AND IN VITRO

Measuring Energy Sources in vivo
Calorimetry

\[ VO_2 = VO_{2i} - VO_{2o} \]
\[ VC_02 = VC_{2i} - VC_{2o} \]
\[ RER = \frac{VC_{2i}}{VO_{2i}} \]

Promethion Cages
Sable Systems International

Maastricht Instruments BV Dual Chamber Whole Body Calorimeter

Cosmed Desktop Metabolic System for Indirect Calorimetry with Mask
Measuring Energy Sources in vivo
Respiratory Exchange Ratio = Respiratory Quotient

The source of energy dictates the amount of O₂ required to oxidize the carbons and hydrogens into CO₂

\[ RER = \frac{VCO_2}{VO_2} \]

Carbohydrate (Glucose)
\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + 38ATP \]
\[ RER = \frac{6CO_2}{6O_2} = 1 \]

Fat (Palmitate)
\[ C_{16}H_{32}O_2 + 23O_2 \rightarrow 16CO_2 + 16H_2O + 129ATP \]
\[ RER = \frac{16CO_2}{23O_2} = 0.7 \]


Measuring Energy Sources in vivo
Respiratory Exchange Ratio = Respiratory Quotient

Breakfast A

Breakfast B

Regular Diet
HFD
HFD + PIO

Measuring substrate preference in vitro
Seahorse technology – standard use

Measuring substrate preference in vitro
Seahorse technology – XF Glycolysis Stress Test Kit
Measuring substrate preference in vitro
Seahorse technology – XT Mito Fuel Flex Test

**WHY DOES IT MATTERS?**

Dependency is the measurement of a cell's requirement for a specific fuel to meet metabolic demand. Capacity is the measurement of their ability to use a specific fuel to meet energy demand. Flexibility is the difference between the amount they need and the amount they could use (capacity minus dependency).
Metabolic flexibility is impaired in individuals with type 2 diabetes

- Metabolically flexible
- Metabolically inflexible (T2D)

Metabolic flexibility is enhanced in athletes

- Trained subjects more effectively decrease glucose uptake and increase fatty acid oxidation than untrained individuals.
- Healthy trained muscle can rapidly switch between fuels
Substrate preference regulates immune cell polarization

Stimulated lymphocytes and macrophages engage in glycolysis and glutaminolysis → augmented rates of glycolytic flux and regeneration of NAD⁺. → biosynthesis of nucleotides, amino acids, and lipids

LPS-stimulated monocytes switch from OXPHOS to glycolysis → increased glucose consumption, lactate production, and NAD⁺/NADH ratio

Fatty acid transport protein 1 knockout mice fed high-fat diets showed an increased proinflammatory phenotype and worsened metabolic syndrome

Substrate preference regulates immune cell polarization

Modulation of substrate preference is crucial for proliferation/differentiation


Metabolic flexibility in cancer

Cancer cells typically exhibit:
1. deregulated uptake of glucose and amino acids
2. use of opportunistic modes of nutrient acquisition
3. use of glycolysis/TCA cycle intermediates for biosynthesis and reduced NADPH production
4. increased demand for nitrogen
5. alterations in metabolite-driven gene regulation
6. specialized metabolic interactions with the microenvironment

→ Reliance of glycolysis is highly variable depending on the cancer cell line
→ Metastatic phenotype suggests that tumor cells do not become metabolically hardwired but remain able to reroute metabolism to adapt to their phenotype and the newly acquired environment

Glycolysis in cancer cells

It is misleading to use glucose uptake and lactate production alone to estimate aerobic glycolysis. The increased glycolysis might be a reflection of the cells adapting to their environment.
Metabolic flexibility in cancer

Constitutive activation of PI3K/Akt signalling addicts tumour cells to glucose by interfering with the induction of fatty acid oxidation when glucose is withdrawn.


Metabolic flexibility in cancer

c-Myc addicts cells to glutamine by preventing them from supplying the tricarboxylic acid cycle using other nutrients.

Metabolic flexibility in cancer

Metabolic gene expression of cancer cells more closely resembles that of the parental tissue than it does of other cancers.

Where tumour cells reside along the spectrum of metabolic flexibility/rigidity may well determine their sensitivity to therapy.


ASSOCIATION BETWEEN METABOLIC DISEASES AND CANCER

Epidemiology
WHO facts

**Diabetes mellitus**
- High blood glucose kills about 3.4 million people annually
- 415 million people have diabetes globally
- Cost >$1.3 trillion/year globally
- Main risk factors for diabetes are behavioural and dietary risks: high body mass index, unhealthy diet, physical inactivity, tobacco use
- Type 2 diabetes is prevented or delayed by modifying diet and increasing physical activity

**Cancer**
- Cancer killed 9.6 million people worldwide in 2018
- Second leading cause of death globally
- Cost >$1.2 trillion/year globally
- Main risk factors for cancer are behavioural and dietary risks: high body mass index, low fruit and vegetable intake, physical inactivity, tobacco and alcohol use
- Between 30% and 50% of cancer deaths could be prevented by modifying or avoiding key risk factors

Both are on the rise!
Prevalence of overweight
Body Mass index ≥ 25 kg/m², ages 18+, males, 2014

Cancers associated with Overweight and Obesity

- **Endometrial cancer**: 2-7 times as likely
- **Esophageal adenocarcinoma**: 2-4 times as likely
- **Gastric cardia cancer**: twice as likely
- **Liver cancer**: twice as likely; Association stronger in men.
- **Kidney cancer**: twice as likely
- **Multiple myeloma**: slight increase in risk (10-20%)
- **Meningioma**: risk increased by 20-50%
- **Pancreatic cancer**: risk increased by 50%
- **Colorectal cancer**: slightly more likely (about 30%).
- **Gallbladder cancer**: slight increase in risk (about 20-60%). Association greater in women.
- **Breast cancer**: In postmenopausal women, higher BMI is associated with a modest increase in risk (20-40%). In premenopausal women, overweight and obesity are associated with a 20% decreased risk. Obesity is also a risk factor for breast cancer in men.
- **Ovarian cancer**: slight increase (5%) in risk.
- **Thyroid cancer**: slight increase in risk (10%)
AIMS/HYPOTHESIS: Diabetes has been shown to be a risk factor for some cancers. Whether diabetes confers the same excess risk of cancer, overall and by site, in women and men is unknown.

METHODS: A systematic search was performed in PubMed for cohort studies published up to December 2016. Selected studies reported sex-specific relative risk (RR) estimates for the association between diabetes and cancer adjusted at least for age in both sexes. Random-effects meta-analyses with inverse-variance weighting were used to obtain pooled sex-specific RRs and women-to-men ratios of RRs (RRRs) for all-site and site-specific cancers.

RESULTS: Data on all-site cancer events (incident or fatal only) were available from 121 cohorts (19,239,302 individuals; 1,082,592 events). The pooled adjusted RR for all-site cancer associated with diabetes was 1.27 (95% CI 1.21, 1.32) in women and 1.19 (1.13, 1.25) in men. Women with diabetes had ~6% greater risk compared with men with diabetes (the pooled RRR was 1.06, 95% CI 1.03, 1.09). Corresponding pooled RRRs were 1.10 (1.07, 1.13) for all-site cancer incidence and 1.03 (0.99, 1.06) for all-site cancer mortality. Diabetes also conferred a significantly greater RR in women than men for oral, stomach and kidney cancer, and for leukaemia, but a lower RR for liver cancer.

CONCLUSIONS/INTERPRETATION: Diabetes is a risk factor for all-site cancer for both women and men, but the excess risk of cancer associated with diabetes is slightly greater for women than men. The direction and magnitude of sex differences varies by location of the cancer.
Cancers associated with type 2 diabetes

Meta-analyses, 2005-2011

- Higher incidence of various types of cancer among individuals with diabetes, particularly type 2 diabetes
- People with diabetes have poorer survival than non-diabetics after a diagnosis of cancer.
- Association with diabetes varies in important respects between cancer types


Association of metabolic diseases with cancers

Biologic plausibility

- Hyperglycemia (i.e., high glucose levels). Availability of glucose fuels cancer cells that typically rely on glucose for metabolism.
- Chronic inflammation. Diabetes and obesity are associated with chronic activation of the immune system in tissues promoting a tumour-prone environment.
- Hyperinsulinemia (i.e., high insulin levels). Insulin promotes the growth of cancer cells, which typically express the insulin receptor.
- In obese individuals, adipose tissue produces excess amounts of estrogen, high levels of which have been associated with increased risks of breast, endometrial, ovarian, and some other cancers.
- Fat cells produce hormones regulating cell growth. Leptin promotes cell proliferation, while adiponectin may have antiproliferative effects.

Association of metabolic diseases with cancers

**Biologic plausibility - hyperglycemia**

Cancer cells rely on glucose for metabolism, hyperglycemia provides extra energy.

Hyperglycemia activates many pathways related to cell proliferation and apoptosis. High glucose decreases E-Cadherin, stimulated epidermal growth factor signalling, modulates the AMPK/mTOR/S6 and MAPK pathways, etc.

Hyperglycemia damages tissues through repeated changes in glucose metabolism and accumulation of glycated biomolecules (AGE).

RAGE expression is associated with metastatic ability of pancreatic cancer cells and with glioma growth and metastasis through MAPK activation and MMP-2/9 induction.

**BUT** Clinical trials of glucose-lowering therapies in type 2 diabetes has shown no diminution of cancer risk.


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Association of metabolic diseases with cancers

**Biologic plausibility – Chronic inflammation**

- Diabetes and obesity are associated with chronic activation of the immune system in tissues promoting a tumour-prone environment.
- Production of free radicals can disrupt signaling and damage DNA.
- IL-6 and TNF-α induce genes promoting proliferation and inhibiting apoptosis by upregulating the transcription factor, NF-κB.
- Elevated levels of IL-6, TNF-α, and C-reactive protein linked to greater risks for colorectal and breast cancer.
- Chronic local inflammation induced by gastroesophageal reflux is a cause of esophageal adenocarcinoma.
- Hepatitis are risk factors for different types of liver cancer.

Association of metabolic diseases with cancers

**Biologic plausibility - estrogen**

- In obese individuals, adipose tissue produces excess amounts of estrogen, high levels of which have been associated with increased risks of breast, endometrial, ovarian, and some other cancers.
- Estrogens seem to support tumor development and progression through direct effects on induction of cellular proliferation and inhibition of apoptosis via ER-α agonism
- Secretion of vascular endothelial growth factor and angiogenesis

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Association of metabolic diseases with cancers

**Biologic plausibility - adipokines**

- Fat cells produce hormones regulating cell growth.
- Leptin promotes cell proliferation through the STAT3 pathway
- Adiponectin has antiproliferative effects through AMPK

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Association of metabolic diseases with cancers

**Biologic plausibility - Hyperinsulinemia**

- In obesity and diabetes, the pancreas oversecretes insulin to compensate for insulin resistance.
- Insulin promotes the growth of cancer cells, which typically express the insulin receptor.
- Chronic hyperinsulinemia reduces IGF binding proteins which result in increased tissue availability of both IGF-I and IGF-II.
- Interfering ratios between insulin and IGF-1 can manipulate tumorigenesis in animal models, suggesting that insulin/IGF signaling positively contributes to tumorigenesis in obese animals.
- Ras/Raf/MAPK and PI3K/Akt/mTOR pathways


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Association of metabolic diseases with cancers

**Regulatory mechanisms**

PREVENTION OF METABOLIC DISEASES AND CANCERS

Association of metabolic diseases with cancers
Diet regulates (almost) everything

- Diet
  - hypercaloric
  - high saturated fat
  - high refined food
  - low fiber

- Obesity
- Diabetes

- Chronic inflammation
- Hyperinsulinemia
- Hyperglycaemia
- Adipokines
- Sex hormones

Cancer

Adapted from Scappaticcio et al. Endocrine. 2017 May;56(2):231-239
Caloric restriction and cancer

Animal studies

<table>
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<tr>
<th>Study</th>
<th>RR (95% CI)</th>
<th>Weight, %</th>
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Prevalence of physical inactivity

Moderate intensity $\geq$150 min/week, ages 18+, males 2010

Data Source: World Health Organization

World Health Organization

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Exercise & diet interventions prevent the development of diabetes

BMI > 24, impaired glucose tolerance (pre-diabetes). Randomly assigned to one of three interventions: standard lifestyle recommendations plus metformin (Glucophage) at a dose of 850 mg twice daily, standard lifestyle recommendations plus placebo twice daily, or an intensive program of lifestyle modification. Lifestyle intervention was healthy low-calorie, low-fat diet and physical activity of moderate intensity, such as brisk walking, for at least 150 minutes per week.

CONCLUSIONS. Lifestyle changes and treatment with metformin both reduced the incidence of diabetes in persons at high risk. The lifestyle intervention was more effective than metformin.

BUT the goal was at least a 7 percent weight loss with a drastic diet in addition to exercise...

Exercise is associated with lower cancer mortality

What can we do?

- Education and Prevention: adjust diet, ↑exercise, ↓smoking, ↓alcohol
- Policies on environmental factors: pollution, junk food
- Research and Treatment: more precise therapies

Would reduce the incidence of both metabolic disease and many cancers

References

- Finley & Thompson. The Metabolism of Cell Growth and Proliferation. The Molecular Basis of Cancer (Fourth Edition) 2015, Pages 191-208.e2
### Doctoral education - VT19

**Experimental techniques in study of metabolic and endocrine disorders**

<table>
<thead>
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<td>Programme</td>
<td>Metabolism och endokrinologi</td>
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<tr>
<td>Language</td>
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<td>Date</td>
<td>2018-11-26 -- 2018-11-30</td>
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<td>Purpose of the course</td>
<td>This course will enable the doctoral student to acquire the necessary knowledge to address experimentally key points of metabolic characterization of experimental models in diabetes research.</td>
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<td>Intended learning outcomes</td>
<td>After the course the students will be able i) to measure glucose transport in isolated rodent skeletal muscle; ii) to measure lipolysis in isolated adipocytes; iii) to dissect out mouse pancreatic islets and measure the insulin release; iv) to judge and analyze obtained data. The students will also be able to describe the possibilities and limitations of the above techniques.</td>
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<tr>
<td>Contents of the course</td>
<td>The course is laboratory based, aiming to give all participants hands on experience with isolation of pancreatic islets, skeletal muscle and adipose tissue. Techniques for measurement of glucose transport in isolated rodent skeletal muscle, of lipolysis in isolated adipocytes, and for studying insulin release from pancreatic islets will be covered. Theoretical and practical considerations will be presented and discussed.</td>
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<td>Teaching and learning activities</td>
<td>The course meets for five days full time, including three full day laboratory practical sessions. The first day will consist of several lectures to give a background to the metabolic questions which will be addressed in the practical part of the course. Our aim is to provide the student with a hands on experience of each technique covered. In order to achieve this, for the laboratory work the course participants will be subdivided into smaller groups.</td>
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<td>Literature and other teaching material</td>
<td>Scientific publications covering the techniques covered will be distributed at the start of the course.</td>
</tr>
<tr>
<td>Course responsible</td>
<td>Alexander Chibalin, <a href="mailto:Alexander.Chibalin@ki.se">Alexander.Chibalin@ki.se</a></td>
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