Nutrition in Metabolic Syndrome

Module 24.5

Molecular Aspects of the Metabolic Syndrome

Learning Objectives

- What is meant by the metabolic syndrome;
- Which function does leptin have;
- How does insulin resistance develop;
- Which diabetic complications could occur and what are the causes for their development;
- How to synthesize O- and N-linked glycoproteins;
- Which functions and special characteristics do the enzymes for O-GlcNAc addition and removal have.

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1. Definition and prevalence of the metabolic syndrome
2. Pathogenesis
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6. Diseases of the metabolic syndrome - Diabetes mellitus type 2 and insulin resistance
7. Diabetic complications and advanced glycation end products (AGEPs)
8. Glycosylation
9. O-GlcNAc

Key Messages

- The metabolic syndrome is a polygenetic disease;
- Obesity (Body Mass Index is higher than 30) is the common cause of high triglycerides;
- Leptin regulates energy balance;
- Renin-angiotensin-system is more active, if subjects are obese;
- Hyperglycaemia causes diabetic complications by increased formation of advanced glycation end products (AGEPs);
- Glycosylation is the process of addition of saccharides to proteins and lipids;
- Most of proteins synthesized in the rough ER undergo glycosylation;
- O-GlcNacylation occurs in the nucleus and cytoplasm;
- Altered O-GlcNacylation may contribute to the development of metabolic syndrome.
1. Definition and prevalence of the metabolic syndrome

**Metabolic syndrome - definition:**

A clustering of cardiovascular risk factors that include elevated blood pressure, dyslipidemia (high triglycerides and low high-density lipoproteins - cholesterol (HDL-C)), impaired glucose metabolism with insulin resistance and obesity (1).

- In 1988 defined by Reaven;
- called variously “syndrome X”, “insulin resistance syndrome”, “dysmetabolic syndrome” and “Reaven-syndrome”.

For the **diagnosis** one disease in the carbohydrate metabolism and two other diseases are necessary. An impaired effect of the hormone insulin is always present.

### The WHO and NCEP definitions of the metabolic syndrome

<table>
<thead>
<tr>
<th>Modified WHO definition</th>
<th>NCEP definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hyperinsulinemia</strong> (upper quartile of the non-diabetic population) OR fasting plasma glucose ≥ 7.0 mmol/l AND at least <strong>two</strong> of the following:</td>
<td>At least <strong>three</strong> of the following:</td>
</tr>
<tr>
<td>• Abdominal obesity</td>
<td>• Fasting plasma glucose ≥ 6.1 mmol/l</td>
</tr>
<tr>
<td>• Dyslipidemia (serum triglycerides ≥ 1.70 mmol/l OR Men: HDL cholesterol &lt; 0.9 mmol/l, Women: HDL cholesterol &lt; 1.1 mmol/l)</td>
<td>• Abdominal obesity</td>
</tr>
<tr>
<td>• Hypertension (blood pressure ≥ 140/90 mmHg or on medication)</td>
<td>• Serum triglycerides ≥ 1.70 mmol/l</td>
</tr>
<tr>
<td>Abdominal obesity</td>
<td>• HDL cholesterol: Men: &lt; 1.0 mmol/l Women: &lt; 1.3 mmol/l</td>
</tr>
<tr>
<td><em>Definition 1:</em> Men: WHR &gt; 0.90 or BMI ≥ 30 Women: WHR &gt; 0.85 or BMI ≥ 30</td>
<td>• Blood pressure ≥ 130/85 mmHg or on medication</td>
</tr>
<tr>
<td><em>Definition 2:</em> Men: waist girth ≥ 94 cm Women: waist girth ≥ 80 cm</td>
<td>Abdominal obesity</td>
</tr>
<tr>
<td><em>Definition 1:</em> Men: waist girth &gt; 102 cm Women: waist girth &gt; 88 cm</td>
<td><em>Definition 2:</em> Men: waist girth &gt; 94 cm</td>
</tr>
</tbody>
</table>

**WHO 1999 and NCEP 2001; JAMA; 285; 2486-2497**

WHO = World Health Organization; NCEP = National Cholesterol Education Program;

In the original WHO definition, insulin resistance in the top 25% of the population as measured by the euglycemic hyperinsulinemic clamp was used instead of hyperinsulinemia. Microalbuminuria was also included in addition to abdominal obesity, dyslipidemia and hypertension.
The prevalence of the metabolic syndrome ranges depend on countries and ethnic groups from 1% to 39%. But in all countries the prevalence increases rapidly with age. The following table gives an overview of studies which investigated the prevalence of the metabolic syndrome (24).

<table>
<thead>
<tr>
<th>Study and Area</th>
<th>Study Population</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hulthe et al., 2000; Arterioscler Thromb Vasc Biol; 20; 2140-7 Gothenborg area Sweden</td>
<td>362 58-year-old men free of cardiovascular disease and hypertensive medication</td>
<td>WHO definition: 16%</td>
</tr>
<tr>
<td>The Third National Health and Nutrition Examination Survey, 2002; JAMA; 287; 356-9 United States</td>
<td>8608 men and women age ≥ 20 years, studied 1988-1994</td>
<td>WHO definition: 24%  NCEP definition: 25%</td>
</tr>
<tr>
<td>European Group for the Study of Insulin Resistance, 2002; Diabetes Metab; 28; 364-76 8 European countries</td>
<td>8200 men and 9363 women</td>
<td>WHO definition: 7%–36% for men 40–55 years old depending on the country; for women of the same age: 7%–22%</td>
</tr>
<tr>
<td>Kuopio Ischaemic Heart Disease Risk Factor Study, 2002; Am J Epidemiol; 156; 1070-7 Eastern Finland</td>
<td>1005 42-60 year-old men studied 1984-1989</td>
<td>WHO definition: 21%  NCEP definition: 14%</td>
</tr>
<tr>
<td>Turkish Adult Risk Factor Study, 2002; Arteriosclerosis;165; 285-92 Turkey</td>
<td>2398 men and women, mean age 49.1 ± 13 years</td>
<td>NCEP definition: 27% of men and 39% of women</td>
</tr>
<tr>
<td>Women`s Health Study, 2003; Circulation; 107; 391-7 United States</td>
<td>14719 apparently healthy women age 45 and older participating in an ongoing trial of aspirin and vitamin E in primary CVD prevention</td>
<td>NCEP definition: 24%</td>
</tr>
<tr>
<td>Bruneck study, 2003; Diabetes Care; 26; 1251-7 Northern Italy</td>
<td>888 40-79-year-old men and women</td>
<td>WHO definition: 34%  NCEP definition: 18%</td>
</tr>
<tr>
<td>Al-Jawati et al., 2003; Diabetes Care; 26; 1781-5 Oman</td>
<td>1419 urban men and women age 20 years and more</td>
<td>NCEP definition: 19.5% of men and 23% of women</td>
</tr>
<tr>
<td>Grupta et al., 2003; Diabetes Res Clin Pract; 61; 69-76 India</td>
<td>1091 urban dwellers age 20 years and more</td>
<td>NCEP definition: 7.9% of men and 17.5% of women</td>
</tr>
<tr>
<td>West Of Scotland Coronary Prevention Study, 2003; Circulation; 108; 414-9 West Scotland</td>
<td>6595 non-diabetic men without CVD (age 55.1± 5.5) who participated in a trial of pravastatin in cardiovascular primary prevention</td>
<td>NCEP definition: 26.2%</td>
</tr>
</tbody>
</table>

Approximately 47 million (24%) of adult Americans have the metabolic syndrome. Over age 60 years, the prevalence raises to 44% (25).
2. Pathogenesis

Insulin resistance is the key factor for the pathogenesis of the metabolic syndrome. This condition is influenced by a complex interplay between multiple genetic variations interacting with numerous environmental factors (27).

On the top of the following diagram, environmental and genetic factors and their interactions contribute to the pathogenesis of the metabolic syndrome. Overweight, especially in the presence of environmental and genetic risk factors, leads to abdominal obesity and ectopic fat deposition with consequent insulin resistance.

At the bottom you see end-stage consequences of the metabolic syndrome (24).

Also abnormalities in serum uric acid concentration are found in patients with insulin resistance. Maybe insulin enhances renal tubular sodium reabsorption, which results in reduced uric acid clearance. This suggests that uric acid levels may also provide insight into identifying individuals with the metabolic syndrome (26).

Obesity is also associated with over expression of tumour necrosis factor-alpha (TNF-α). TNF-α can in turn cause insulin resistance in obese subjects. Insulin has an anti-inflammatory action. These interactions remain to be elucidated (28).
3. Diseases of the metabolic syndrome - Adiposity and Leptin

Definition: An abnormal increase of body fat mass (BMI ≥30) Obesity is a causative factor in the development of metabolic syndrome.

Health Risks of Obesity
- High blood pressure and stroke are twice as common in obese people;
- Evidence is strong that obesity increases the risk of breast cancer (after menopause), womb cancer and kidney cancer;
- Obesity may also increase the risk of colon cancer;
- Gall bladder disease is three times as likely to occur in middle-aged obese women;
- Diabetes is four times more common in middle-aged obese people than in middle-aged people of normal weight;
- Coronary heart disease is twice as common in obese men under 45;
- Osteoarthritis is more painful and less easily treatable if the person is obese;
- Severe obesity may cause shortage of breath, varicose veins, backache and even psychological problems.
Leptin:
- is a small 16 kDa protein (167 amino acids);
- mainly produced in adipocytes;
- encoded by the ob-gene (ob = obese), isolated in 1994 by Zhang et al.;
- regulates energy balance by acting on the hypothalamus to reduce food intake and to increase energy expenditure via sympathetic activation;
- acts through the leptin receptor, a single-transmembrane-domain receptor of the cytokine receptor family, which is found in many tissues in several alternatively spliced forms;
- in the hypothalamus there is a leptin receptor with high affinity for leptin;
- the long form of leptin receptor is expressed in pancreatic β-cells;
- subjects with heterozygous leptin gene mutations have low circulating leptin levels and increased body adiposity;
- defects in leptin production cause severe hereditary obesity in rodents and humans;
- leptin insensitivity in brain can also cause adiposity; leptin concentration has to be higher then, so that the signal can be recognized by the hypothalamus; it is not always a deficiency of leptin which causes obesity;
- can increase insulin sensitivity, this action appears to be mediated by direct and indirect (CNS) effects to activate AMP kinase and increase muscle fatty acid oxidation;
- can inhibit insulin secretion by activating with ATP-dependent potassium channels or via interactions with the cAMP protein kinase A signalling pathway perhaps by activating phosphodiesterase B3.
4. Diseases of the metabolic syndrome - Hypertension

**Definition:**
systolic blood pressure > 140 mmHg;
diastolic blood pressure > 90 mmHg

<table>
<thead>
<tr>
<th>normotension</th>
<th>systolic blood pressure</th>
<th>&lt; 140 mmHg</th>
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<tbody>
<tr>
<td></td>
<td>diastolic blood pressure</td>
<td>&lt; 90 mmHg</td>
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</table>

<table>
<thead>
<tr>
<th>hypotension</th>
<th>systolic blood pressure</th>
<th>&lt; 100 mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>diastolic blood pressure</td>
<td>&lt; 60 mmHg</td>
</tr>
</tbody>
</table>

15% of the people living in industrialized countries have hypertension. 90% of these have the essential form. Obesity is the most common cause of essential hypertension. It is suggested that excess renal sodium reabsorption and a hypertensive shift of pressure natriuresis play a major role. Sympathetic activation appears to mediate at least part of the obesity-induced sodium retention and hypertension since adrenergic blockade or renal denervation markedly attenuates these changes.

Recent observations suggest that leptin and its multiple interactions with neuropeptides in the hypothalamus may link excess weight gain with increased sympathetic activity. Transgenic mice overexpressing leptin also develop hypertension. Maybe the renal sympathetic effects of leptin may depend on interactions with other neurochemical pathways in the hypothalamus, including the melanocortin-4 receptor.

The Renin-Angiotensin system is more active if subjects are obese and have hypertension. There is also a direct association between hyperinsulinemia and hypertension because insulin causes increased reabsorption of sodium in the tubules of kidney. An increased supply of sodium causes increased blood pressure because the intracellular higher concentration of sodium inhibits the calcium-sodium-exchange. The result is an increase in the calcium-concentration in the vascular muscle tissue that leads to the increased muscle tonus (3). Furthermore high insulin levels stimulate the sympathetic nervous system and increase angiotensin II production (25).

5. Diseases of the metabolic syndrome - Dyslipidemia

**Definition:** HDL-C (high density lipoprotein - cholesterol) low and LDL-C (low density lipoprotein - cholesterol) and triglycerides high.

Obese people have generally a higher intake of saturated fatty acids and cholesterol. Saturated fatty acids cause a lower activity of the LDL-receptor, what leads to a slower intake of LDL in cells. LDL-C in serum is then higher so that LDL binds the scavenger-receptor. This receptor mediates the storage of cholesterol in skin, in walls of blood-vessels and in macrophages. This is a risk factor for atherosclerosis.

Obese people have increased levels of free fatty acids. This has the following effects:
- Free fatty acids (FFA) can reduce the hepatic insulin clearance;
- FFA can increase the synthesis of glucose in the liver;
- FFA can lead to an impaired glucose utilization in the skeletal muscle;
- FFA can increase the synthesis of VLDL (4).
6. Diseases of the metabolic syndrome - Diabetes mellitus type 2 and insulin resistance

Non-insulin-dependent diabetes mellitus (NIDDM), because insulin treatment is not always needed
Common cause: Insulin resistance with a relative lack of insulin; 90% of the patients are obese; A lack of insulin causes hyperglycaemia, glucosuria and increased gluconeogenesis.
Main characteristics:

hyperinsulinemia
↓
insulin resistance
↓
the amount of insulin receptors decreases and
the binding of the hormone is impaired

- Insulin resistance - the tissues such as muscles don't respond fully to the actions of insulin, so can not make use of glucose in the blood. The pancreas response by producing more insulin and the liver releases more glucose to try to increase the amount of glucose available. The pancreas is then not able to produce enough insulin and the tissues become resistant to insulin;
- Usually develops over 40 years of age;
- Obese people are more at risk of type 2 diabetes and also higher risk for people who are an "apple-shape" - with lots of fat around the abdomen;
- Ketones are in urine, ketones indicate there is not enough insulin to prevent the mobilization of fat;
- Hyperlipidemia, because of the greater synthesis rate of lipoproteins;
- Hyperglycaemia seems to cause raised levels of atherogenic cholesterol-enriched apolipoprotein B-containing remnant particles (5).

More than 19 million adults in the United States and 150 million worldwide have diabetes; by the year 2025 the WHO projects more than 300 million cases worldwide (23).

7. Diabetic complications and AGEP

- Diabetes-specific microvascular pathology in the retina, renal glomerulus and peripheral nerve;
- Arteriosclerotic macrovascular disease affecting arteries that supply the heart, brain and lower extremities;
- Higher risk of myocardial infarction, stroke and limb amputation.

Hypothesis: Hyperglycaemia causes diabetic complications by increased advanced glycation end products formation (AGEPs). AGEPs are found in increased amounts in diabetic retinal vessels and renal glomeruli. They were originally thought to arise from non-enzymatic reactions between extracellular proteins and glucose. Accumulation of AGEP-cross linked proteins throughout life is a general phenomenon of ageing. They are markers of protein ageing. AGEPs are protein modifications: they are formed by a complex cascade of dehydration, oxidation and cyclisation reactions, subsequent to a non-enzymatic reaction of sugars with amino group of proteins. AGEPs are Maillard-products. Intracellular hyperglycaemia is the primary initiating event in the formation of intracellular and extracellular AGEPs because the rate of AGE formation from glucose is orders of magnitude slower than the rate of AGE formation from glucose-derived dicarbonyl precursors generated intracellularly.

Effects of AGE precursors on cells:
AGEP precursors bind the AGEP receptors (RAGE = receptor for advanced glycation end products) on
- endothelial cells;
- mesangial cells;
- macrophages;
inducing receptor-mediated production of reactive oxygen species.
Inhibitors of Maillard-reaction are currently being assessed in clinical trials for the treatment of diabetic complications (6).

There is a growing evidence of a link between aberrant O-GlcNAc modification and diabetes. One of the hallmarks of type 2 diabetes is hyperglycaemia associated with an inability of insulin to trigger appropriate glucose uptake (insulin resistance). Glucose flux through the hexosamine pathway has been linked to the onset of insulin resistance (7). Increased levels of extracellular glucose and glucosamine lead to elevated intracellular O-GlcNAc modification of proteins in skeletal muscle and in pancreatic β-cells (8).

In muscle cells several postreceptor insulin signalling events are dampened under hyperglycaemic conditions and reduced IRS-1 and IRS-2 (insulin receptor substrate) signalling are associated with their increased O-GlcNAc modification and decreased phosphorylation (9). Thus it is proposed that hyperglycaemia-induced O-GlcNAc modifications perturb normal signalling events required for insulin mediated homeostasis. Because O-GlcNAc levels on proteins appear to be sensitive to flux through the hexosamine biosynthetic pathway, a role as a general sensor of glucose availability can be hypothesized for O-GlcNAc (10).

8. Glycosylation

Definition: Process of addition of saccharides to proteins and lipids

- one of four principal post-translational modification steps in the synthesis of membrane and secreted proteins;
- most of the proteins synthesized in the rough ER undergo glycosylation;
- it is an enzyme-directed site-specific process;
- the donor molecule is an activated nucleotide sugar;
- two different forms exist.
N-linked glycosylation

- All N-linked carbohydrates are linked through N-acetylglucosamine and the amino acid asparagine;
- The N-linked amino acid consensus sequence is Asn-X-Ser or Thr. The middle amino acid cannot be proline (Pro), but any other;
- After attachment once the protein is correctly folded, the three glucose residues are removed from the chain and the protein is available for export from ER;
- The glycoprotein is then transported to the Golgi where removal of further mannose residues may take place;
- Further removal of mannose residues leads to a core structure containing 3 mannose and 2 N-acetylglucosamine residues which may then be elongated with a variety of different monosaccharides including galactose, N-acetylgalactosamine, N-acetylgalactosamine, fucose and sialic acid.

O-linked glycosylation

- Most O-linked carbohydrate attachments to proteins involve a linkage between the monosaccharide N-Acetylgalactosamine and the amino acids serine or threonine;
- Currently there is not an O-linked amino acid consensus sequence (search for article).

Posttranslational modifications like glycosylation play a major role in many biological processes, for example: signal transduction, gene expression and metabolism. Glycosylation is one of the most common posttranslational modifications of proteins in eukaryotes. It affects a wide range of protein functions:
- Protein folding
- Protein secretion
- Serum half-life
- Biomolecular recognition (11).

![Fig. 12](image1.png)

N-linked glycosylation

to the amide nitrogen of asparagine side chains

- is required for some proteins for proper folding
- occurs in eukaryotes, widely in archa and very rarely in prokaryotes
- two major types of N-linked saccharides: high-mannose oligosaccharides and complex oligosaccharides

SYNTHESIS:
- a 14-sugar precursor is first added to the asparagines in the polypeptide chain of the target protein
- this precursor is common to most eukaryotes and contains 3 glucose, 9 mannose, 2 N-acetylglucosamine molecules
- a complex set of reactions attaches this branched chain to a carrier molecule called dolichol
- it is then transferred to the appropriate point on the polypeptide chain as it is translocated into the ER lumen

![Fig. 13](image2.png)

O-linked glycosylation to the hydroxy oxygen of serine and threonine side chains by the enzyme UDP-N-acetyl-D-Galactosamine:polypeptide N-cetylgalactosaminyltransferase

- is important for some protein such as proteoglycans
- occurs at a later stage during protein processing, probably in the Golgi apparatus

SYNTHESIS:
- addition of N-acetyl-galactosamine followed by the addition of other carbohydrates such as sialic acid and galactose

![Fig. 14](image3.png)

O-linked glycoprotein  N-linked glycoprotein

Protein folding  Protein secretion
Serum half-life  Biomolecular recognition (11)
9. O-GlcNAc

O-GlcNAc was discovered by Torres and Hart in 1984. 51 years ago the phosphorylation was already get to know (12). The addition of a single O-linked N-acetylglucosamine - the Ser(Thr)-O-GlcNAcylation is dynamic and abundant analogous to phosphorylation (13).

O-GlcNAcylation occurs in the nucleus and cytoplasm. Altered O-linked GlcNAc metabolism may occur in the development of neurodegenerative disorders, diabetes mellitus, and cancer.

All of the O-GlcNAcylated proteins are also phosphoproteins. A reciprocal relationship between these modifications exists (14). Both are dynamic: O-GlcNAc and phosphate are added or removed in minutes. The O-GlcNAc half-life is much shorter than that of the modified polypeptide chain.

The sites of O-GlcNAc modification are often the same or adjacent to sites of phosphorylation, suggesting a role in regulation analogous to or competition with phosphorylation.

Thus, it is hypothesized that O-GlcNAc regulates the functions of proteins, either exclusively or in concert with phosphorylation.

Reciprocity between O-GlcNAc and phosphorylation, this so-called “yin-yang” relationship has been shown at both the global cellular protein level and at specific sites on particular proteins.

For example inhibitors of kinases increase the overall level of O-GlcNAc modified proteins (15). Furthermore the enzymes that catalyze the cycling of O-GlcNAc onto and of proteins are analogous to those that add and remove phosphates (kinases and phosphatases) (16). O-GlcNAcylation and phosphorylation can compete for the same site or adjacent sites like in the case of RNA Pol II (17).
Enzymes for O-GlcNAc modification and removal

Uridine diphospho-N-acetylglucosamine: polypeptide β-N-acetylglucosaminytransferase (OGT)
- Catalyzes the addition of O-GlcNAc on proteins;
- Was originally purified in 1992 from rat liver;
- Molecular weight: 340 kDa approximately (18);
- Human and rat sequence are nearly 100% homologous;
- The gene of OGT resides on the X chromosome and is necessary for stem cell viability (19);
- The protein is composed of two 110 kDa polypeptides and one 78 kDa polypeptide;
- Each polypeptide appears to be composed of two domains;
- N-terminus contains 11.5 tetratricopeptide repeats (TPR). These repeats are thought to be involved in mediating protein-protein interactions (20);
- C-terminus appears to be the catalytic domain with a putative UDP-GlcNAc binding site;
- There are multiple isoforms of OGT, one splice variant is targeted to mitochondria;
- OGT is O-GlcNAc modified and tyrosine-phosphorylated;
- A deletion of 100 amino acids from the C-terminus results in a catalytic inactive enzyme;
- Understanding the regulation of OGT will be key to future investigation of O-GlcNAc modification (21).

O-GlcNAcase
- Removes O-GlcNAc from proteins;
- Consists of 916 amino acids;
- Purified and characterized by Dong et al., 1994 (22);
- Localization primarily to the cytosol and to a lesser extent to the nucleus;
- Specifically catalyzes the removal of O-GlcNAc from proteins and not GalNAc;
- Is a cytosolic neutral β-N-acetylglucosaminidase;
- Molecular weight: 130 kDa, native O-GlcNAcase activity migrates at 600 kDa, indicating that in the cell, the enzyme may be complexed with proteins like hsp110 for example;
- The gene for O-GlcNAcase resides on the chromosome 10 (10q24);
- C-terminal half contains the O-GlcNAcase activity;
- Very little is known about the regulation of the O-GlcNAcase (16).
O-GlcNAc and its functions

This figure shows that O-GlcNAc is implicated in many cellular processes in the cytosol and in the nucleus (10).

Fig. 18 Abbreviations: phosphatidylinositol-3 kinase (PI-3 kinase), TATA-binding protein-associated factor (p110), c-myc (myc), estrogen receptor b (ERb), and RNA polymerase II (Pol II). Numbers in parentheses are reference numbers. Question marks represent unpublished work and/or speculation on the part of the authors.
The current list of O-GlcNAc-modified proteins is decidedly incomplete, as detection of O-GlcNAc proteins present in soluble cells extracts reveals thousands of proteins that contain this modification.

The following table gives an overview of O-GlcNAcylated proteins:

### Table 1 O-GlcNAcylated proteins

<table>
<thead>
<tr>
<th>Functional Subgroup</th>
<th>Protein</th>
</tr>
</thead>
</table>
| **Chaperones**      | Heat shock protein 27 (HSP27)  
                      | Heat shock cognate 70 (HSC70)  
                      | Heat shock protein 70 (HSP70)  
                      | Heat shock protein 90 (HSP90)  |
| **Chromatin**       | Chromatin-associated proteins |
| **Cytoskeleton**     | Ankyrin G  
                      | Cofilin  
                      | E-cadherin  
                      | Myosin  
                      | Protein band 4.1  
                      | Synapsin  
                      | Talin |
| **Intermediate**    | Keratins 8, 13, 18  
                      | Neurofilaments H, M, L |
| **filaments**       | **Microtubule-based** | a-Tubulin  
                      | Dynein LC1  
                      | Microtubule-associated proteins 2 and 4 (MAP 2 and 4)  
                      | Tau |
| **Other**           | Adenovirus type 2 and 5 fibre proteins  
                      | Assembly protein 3 and 180 (AP-3 and AP-180)  
                      | b-Amyloid precursor protein (b-APP)  
                      | b-Synuclein  
                      | Piccolo  
                      | Plakoglobin |
| **Kinases and adaptor proteins** | Casein kinase II (CKII)  
                      | Glycogen synthase kinase-3b (GSK-3b)  
                      | Insulin receptor substrate 1 and 2 (IRS-1 and IRS-2)  
                      | PI3 kinase (p85) |
| **Metabolic enzymes** | Enolase  
                      | Endothelial nitric oxide synthase (eNOS)  
                      | Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)  
                      | Glycogen synthase (GS)  
                      | Phosphoglycerate kinase (PGK)  
                      | Pyruvate kinase (PK)  
                      | UDP glucose pyrophosphorylase (UGP) |
| **Nuclear hormone receptors** | Estrogen receptor-a and -b (ER-a and ER-b)  
                      | V-erb A |
| **Nuclear pore proteins (NUP)** | Nup 62  
                      | Nup 153, 214, 358  
                      | Nup 180  
<pre><code>                  | Nup 54, 155 |
</code></pre>
<table>
<thead>
<tr>
<th>Category</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatases</td>
<td>Nuclear tyrosine phosphatase p65, Phosphatase 2a inhibitor (I2pp2a)</td>
</tr>
<tr>
<td>Polymerases</td>
<td>RNA Pol II</td>
</tr>
<tr>
<td>Proto-oncogenes</td>
<td>c-Myc</td>
</tr>
<tr>
<td>RNA-binding proteins</td>
<td>40S ribosomal protein S24 (40SrpS24), Elongation factor 1a (EF-1a), Eukaryotic initiation factor 4A1 (4A1)</td>
</tr>
<tr>
<td>Tumour suppressors</td>
<td>Retinoblastoma protein (Rb)</td>
</tr>
<tr>
<td>Viral proteins</td>
<td>Baculovirus gp41 tegument protein, HCMV UL32 (BPP) tegument protein, NS26 rotavirus protein, SV-40 large T antigen, Virion basic phosphoprotein</td>
</tr>
<tr>
<td>Transcription factors</td>
<td>AP-1 (c-fos and c-jun), b-Catenin, CAAT box transcription factor (CTF, NF-1), Cyclic AMP response element-binding protein (CREB), ELF-1 (Ets transcription factor), Enhancer factor 2D (EF-2D), Hepatocyte nuclear factor 1 (HNF-1), KIAA0144, Oct1, NF-kB, OGT-interacting protein 106 (OIP-106), p53, Pancreatic/duodenal homeobox-1 protein (PDX-1, IPF-1, STF-1), PAX-6, Pancreas-specific transcription factor (PTF-1), Human C 1 transcription factor (HCF), Serum-response factor (SRF), Sp1 and Ying yang 1 (YY1)</td>
</tr>
<tr>
<td>Other</td>
<td>Annexin 1, Collapsin response mediator protein-2 (CRMP-2), Elongation initiation factor-2 associated 67 kDa (EF2a p67), GABA receptor interacting protein-1 (GRIF-1) and splice variants, Glut-1 and Glut-4, Nucleophosmin, Peptidyl prolyl isomerase (PPI), Proteosome component C2, O-GlcNAc transferase (OGT), Q04323, UCH homologue, Sec23, human homologue (hhSec23), Ran, Rho GDP dissociation inhibitor 1 (Rho-GDIa), Ubiquitin carboxy hydrolase (UCH)</td>
</tr>
</tbody>
</table>
Abbreviations

O-GlcNAc  O linked N-acetylglucosamine
GalNAc  N-acetylgalactosamine
AGEP  advanced glycation end products
ER  estrogen receptor
ERb  estrogen receptor b
HDL-C  high-density lipoproteins - cholesterol
LDL-C  low density lipoproteins - cholesterol
TNF-α  tumour necrosis factor-alpha
FFA  free fatty acids
VLDL  very low density lipoproteins
RAGE  receptor for advanced glycation end products
IRS  insulin receptor substrate
TPR  tetratricopeptide repeats
PI-3 kinase  phosphatidylinositol-3 kinase

References