Learning Objectives

- What is the statement of the HSP hypothesis;
- Why do GFAT and OGT play a so important role;
- What about the effects of STZ, ALX and PUGNAc;
- Mechanisms through which FFAs can upregulate the HSP.

Contents

1. HSP hypothesis
2. Effects of overexpression/inhibition of GFAT
3. Role of OGT in β-cells and effects of overexpression
4. Effects of STZ, Alloxan and PUGNAc on β-cells and O-GlcNAcylation? Experimental results
5. Regulation of leptin synthesis
6. Effects of FFA on the biosynthesis of hexosamines
7. Oxidative stress
   8. How to prevent metabolic disorders?

Key Messages

- HSP flux regulates leptin secretion;
- In human with NIDDM GLUT4 function and translocation is impaired;
- GFAT can be inhibited by UDP-GlcNAc;
- Elevation of O-GlcNAC levels attenuate insulin signaling;
- Increased fatty acids upregulate the HSP;
- Probably at the level of transcription and translation leptin production is regulated by hexosamines.
Preface

Western societies have shifted to a higher caloric diet and more sedentary lifestyle, the incident of type 2 diabetes has increased to epidemic proportions. Type 2 diabetes has been described as a disease of "chronic overnutrition". While the genesis of type 2 diabetes is still unclear, certain genetic traits predispose individuals for development of the disease when exposed to certain environmental factors, namely chronic nutrient excess and low energy expenditure. The increase in prevalence of metabolic syndrome parallels the increased prevalence in obesity. There is a growing evidence of a link between aberrant O-GlcNAc modification and diabetes. One of the hallmarks of type 2 diabetes is the hyperglycemia associated with an inability of insulin to trigger appropriate glucose uptake (insulin resistance). Glucose flux through the hexosamine pathway (HSP) has been linked to the onset of insulin resistance. Increased levels of extracellular glucose and glucosamine lead to elevated intracellular O-GlcNAc modification of proteins in skeletal muscles and in pancreatic β-cells. In muscle cells several postreceptor insulin signaling events are dampened under hyperglycemic conditions. Reduced insulin receptor substrate 1 and 2 signaling is associated with increased O-GlcNAc modification and decreased phosphorylation. Thus it is proposed that hyperglycemia-induced O-GlcNAc modifications perturb normal signaling 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.

1. HSP hypothesis

Model of the HSP hypothesis

- O-GlcNAc modification of proteins is acting as a nutrient sensor or glucose sensor.

- In this model cells are taking into account their energy levels (glucose and fatty acids for example). This helps the cell to modulate which proteins to produce in that cell.

- Probable O-GlcNAc is an important regulatory modification and is involved in signal cascades.

Fig. 1 Wells and Hart, 2003; FEBS Letters; 546; 154-58

- Excessive concentrations of glucosamine lead to insulin resistance. It is known that suppression of expression of glutamine-fructose-6-phosphate-amidotransferase (GFAT) can block insulin resistance (1);

- Increased free fatty acids can inhibit glycolysis and can increase fructose-6-phosphate levels;

- Hypercaloric intake can be positively correlated with increased flux through the HSP (2);

- Leptin alters nutrient flux such that energy expenditure is favored over energy storage;

- HSP flux regulates leptin secretion: increased levels of hexosamines lead to an increase in leptin release from adipocytes (3);

- In type 2 diabetic patients hyperglycemia and hyperinsulinemia lead to elevated UDP-GlcNAc levels;

- Increased free fatty acids (FFA) upregulate the HSP presumably by inhibiting glycolysis and increasing glucose-6-phosphate levels;

- HSP plays a role in regulating insulin resistance and serving as an energy sensor (increased flux through the HSP resulting in elevated UDP-GlcNAc levels). Hypercaloric intake correlates positively with increased flux through the HSP (2);

- Elevation of O-GlcNAc levels in 3T3-L1 adipocytes, including the O-GlcNAc modification of several key proteins in the insulin signaling pathway (insulin receptor substrate-1 (IRS-1) and β-catenin) directly causes insulin resistance, the hallmark of type 2 diabetes (4).
2. Effects of overexpression/inhibition of GFAT

An overexpression of the rate limiting enzyme of the HSP-GFAT in skeletal muscle and adipose tissue of transgenic mice lead to weight-dependent hyperinsulinemia and insulin resistance. This lead to decreased levels of the insulin-stimulated GLUT4 especially in older transgenic mice. In human with NIDDM GLUT4 function or translocation is impaired (5). These results show that the GFAT plays an important role in the development of insulin resistance. The defects in glucose transport seen in the transgenic mice are very similar to those seen in human type 2 diabetes (6). The GFAT can be inhibited by UDP-GlcNAc in rat adipocytes (end product inhibition) (7). An increased activity of the GFAT caused by glucose and insulin was observed in cultured human skeletal muscle cells. This suggests an important relationship between GFAT activity and the regulation of glucose homeostasis and support the hypothesis that the HSP is a major pathway used by tissues to sense and respond to changes in glucose flux (8).

The cDNA of GFAT was cloned in 1992 and gene coded the enzyme is localized on chromosome 2 (p13). ob/ob mice have a twofold increase in GFAT activity in comparison to lean mice (9). An overexpression of GFAT was also observed in diabetic glomeruli (10). It is suggested that angiotensin II regulates GFAT promoter activity by modulating signaling pathways that include calcium, PKC and tyrosine kinase cascades. Angiotensin II increases the activity of GFAT. Thus it is also suggested that angiotensin II plays an important role in the development of diabetic complications such as vascular and glomerular injury (11).

3. Role of OGT in β-cells and effects of overexpression

In the presence of high intracellular glucose concentrations the activity of OGT (uridine diphospho-N-acetylglucosamine: polypeptide β-N-acetylglucosaminyltransferase) is higher than the activity of O-GlcNAcase. Using an in situ hybridization it is observed that transcripts encoding OGT are present at a very high level in β-cells of the pancreas. Thus it could be suggested that in β-cells the O-GlcNAc metabolism is higher than in other cells and the OGT might have a unique function in β-cells (12).

It has been demonstrated that the ob gene and the levels of leptin are regulated by hexosamines. Some data support the hypothesis that the regulation occurs through the O-glycosylation pathway. OGT transgenic mice have higher serum leptin levels. It is obviously that OGT like GFAT presumably plays a similar important role in insulin- and leptin signaltransduction cascades (13).
4. Effects of STZ, Alloxan and PUGNAc on β-cells and O-GlcNAcylation

Experimental results

Alloxan and streptozotocin are used to create animal models of diabetes. Alloxan is an analog of uracil and streptozotocin (STZ) is a GlcNAc analog. Both cause diabetes by interfering with proteins that bind UDP-GlcNAc or O-GlcNAc. STZ is an irreversible inhibitor of O-GlcNAcase. When isolated islets were exposed to STZ alone, O-GlcNAcase was inhibited and β-cells were destroyed. When STZ-induced O-GlcNAcase inhibition was prevented by GlcNAc, β-cells remained viable. GlcNAc blocks STZ toxicity because it blocks the entering of STZ in β-cells. Glucose, glucosamine and GalNAc cannot prevent β-cell death. Alloxan block STZ-induced increases in β-cell O-glycosylation. This supports the hypothesis that alloxan is an inhibitor of OGT.

Hyperglycemia has the same effect on the β-cells as STZ, however, the effect is reversible and thus less acutely toxic. The chronic β-cell toxicity caused by hyperglycemia-induced (reversible) O-glycosylation exacerbated the very hyperglycemia itself, which in turn causes more β-cell toxicity, resulting in a chronic downhill spiral developing over a period of years. Since the OGT-rich β-cell is the cell type most sensitive it should also be the cell type most responsive to therapeutic manipulation of the pathway (14).

Another inhibitor called PUGNAc (O-(2-acetamido-2-deoxy-D-glucopyranosylidene)amino-N-phenylcarbamate) is an inhibitor of O-GlcNAcase. PUGNAc treatment increases levels of O-GlcNAc and causes insulin resistance in 3T3-L1 adipocytes. PUGNAc inhibition of O-GlcNAcase affects phosphorylation of AKT at Thr-308 and GSK3β at Ser-9. The phosphorylation is inhibited by PUGNAc. PUGNAc-induced insulin resistance is associated with increased O-GlcNAc modification of several proteins including IRS-1 and β-catenin, two important effectors of insulin signaling. These results suggest that elevation of O-GlcNAc levels attenuate insulin signaling and contribute to the mechanism by which increased flux through the HSP leads to insulin resistance (in adipocytes) (4).

5. Regulation of leptin synthesis

It was observed that hexosamine biosynthesis results in increased leptin release and inhibition of hexosamine biosynthesis with DON (6-diazo-5-oxo-L-norleucine), a competitive inhibitor of GFAT, results in a decrease in ob gene expression and thus in a reduced leptin production. Also a significant positive correlation was observed between BMI and UDP-GlcNAc concentration in human sc (subcutaneous) adipose tissue and between leptin and UDP-GlcNAc in humans. These findings support the hypothesis that leptin production is regulated by the hexosamine production in human adipose tissue probably at the level of transcription and translation. Maybe there exist transcription factors specific for the ob gene promoter which are O-GlcNAc modified. This could be the mechanism through which hexosamines regulate leptin production (15).

6. Effects of FFA on the biosynthesis of hexosamines

Increased free fatty acids upregulate the HSP presumably by inhibiting glycolysis and increasing fructose-6-phosphate levels (16). In a study was investigated through which molecular mechanism free fatty acids induce activation of the HSP. Subjects were stimulated with different fatty acids for 20 hours. The results depended on the degree of unsaturation. Palmitate and stearate (saturated fatty acids) resulted in a three- to fourfold increase in mRNA expression of GFAT. Palmitate increased the amount of O-GlcNAc 1.3-fold. Unsaturated fatty acids had little or no effect. Skeletal muscle insulin resistance has been correlated to the increased availability of FFAs. The molecular mechanism for this upregulation is currently unknown but it is clear that there are transcription factors and signaling pathways through which saturated fatty acids induce GFAT gene activation (17). In addition to this increased fatty acids availability generates increased acetyl-CoA which inhibits pyruvate dehydrogenase and thus ultimately the rate of glycolysis. This results in increased accumulation of fructose-6-phosphate and hence increased substrate for the GFAT (2).
7. Oxidative stress

Fat accumulation correlated with systemic oxidative stress in humans and increased oxidative stress underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular wall cells. Fatty acids stimulate the ROS production via NADPH oxidase activation (18). Characteristics of subjects presenting metabolic syndrome are:
- elevated plasma levels of oxidized lipids;
- subnormal levels of low-molecular-weight antioxidants.

8. How to prevent metabolic disorders?

- Avoidance and management of overweight, especially central obesity;
- Promoting the consumption of diets that are low in fat;
- Reduced consumption of saturated fat to less than 7% of total calories;
- Reduced consumption of foods with high glycemic index. Metabolic consequences of carbohydrates depend not only on their quantity but also on their quality. The glycemic response of a given carbohydrate load depends on the food source, which has led to development of the glycemic index, ranking foods by their ability to raise blood glucose levels. Furthermore effects on blood glucose depend on fiber content and type. Lipid-lowering properties were observed for grain products which are rich in soluble fiber, like oat, barley, rye, psyllium. Insoluble fiber, in wheat and corn for example, was found to be inversely associated with diabetes risk (22, 23, 24);
- Reduction in the consumption of refined carbohydrates and sugar (22);
- Reduced consumption of cholesterol to less than 200 mg/day;
- Promoting the consumption of diets that are high in fish to increase the uptake of polyunsaturated fatty acids (especially omega-3 and omega-6 fatty acids) to decrease LDL cholesterol and increase HDL cholesterol;
- Promoting the consumption of unsaturated fats from natural liquid vegetable oils and nuts at the expense of saturated and trans fats;
- Promoting the consumption of diets that are high in fruits and vegetables to enhance the intake of vitamins and to decrease LDL cholesterol;
- Promoting the consumption of diets that are high in starchy carbohydrates;
- Regular physical activity (can also decrease LDL cholesterol);
- Smoking is a risk factor. Smoking adversely affects glycemic control and increases micro- and macrovascular complications;
- In the first year of life breast feeding is very important, because the protein intake per kg body weight is some 55-80% higher in formula fed than in breast fed infants. The high early protein intake can increase later obesity risk (early protein hypothesis). Also the energy supplies of formula fed infants are 10-18% higher (19).

Childhood obesity:
- Reduced television viewing;
- Increased physical activity;
- Increased vegetables and fruits intake;
- Low fat diet;
- Reduced consumption of sugar-sweetened drinks at home and at school (25).

Abbreviations

HSP  hexosamine pathway
HSC  heat shock cognate
GFAT  glutamine: fructose-6-phosphate-amidotransferase
OGT  uridine diphospho-N-acetylglucosamine: polypeptide -N-acetyl-glucosaminyl-transferase
STZ  steptozotocin
ALX  alloxan
PUGNac  O-(2-acetamido-2-desoxy-D-glucopyranosylidene) amino-N-phenylcarbamate
UDP-GlcNAc  N-acetyl -UDP-glucose
IRS  insulin receptor substrate
PKC  protein kinase C
GlcNAc  N-acetyl glucose
DON  6-diazo-5-oxo-L-norleucine
ROS  reactive oxygen species

References

297


