THE OBESE MAN TO OBESE YEAST

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ABSTRAK

OBESITAS MOLEKULER PADA MANUSIA, AVERTEBRATA, DAN KHAMIR

Dalam beberapa tahun terakhir, obesitas telah menjadi salah satu masalah kesehatan utama di negara maju, dengan Amerika Serikat ada di urutan teratas. Meskipun terjadi perbaikan besar pada faktor sanitasi, perawatan kebidanan dan vaksinasi, tetapi usia harapan hidup menurun seiring dengan meningkatnya obesitas. Saat ini, obesitas merupakan epidemi global yang memengaruhi orang dewasa dan anak-anak, dan secara signifikan berhubungan dengan morbiditas dan mortalitas. Epidemi ini disebabkan oleh pergeseran besar penduduk ke pusat-pusat perkotaan, keterlibatan dalam pekerjaan dan aktivitas hidup santai, serta berlimpahnya makanan olahan padat-energi. Gaya hidup modern yang dianut oleh mayoritas penduduk di perkotaan sulit untuk mengurangi obesitas secara signifikan sehingga diperlukan upaya lain seperti terapi secara farmakokinetis dan pemahaman lebih dalam untuk mengendalikan nafsu makan dan asupan makanan. Gaya hidup dan faktor keturunan berperan penting terhadap timbulnya obesitas. Penelitian terhadap mutasi genetika pada manusia dan model organisme memberikan dasar-dasar wawasan tentang proses fisiologis kompleks dalam penguraian metabolisme lemak. Penelitian terbaru dari khamir *Saccharomyces cerevisiae*, cacing *Caenorhabditis elegans*, lalat buah *Drosophila melanogaster* dan ikan zebra *Danio rerio* menunjukkan bahwa organisme tingkat rendah seperti metazoa memiliki atribut unik yang dapat membantu mengidentifikasi, menyelidiki, bahkan memvalidasi target baru dalam dunia farmasi untuk penyakit-penyakit yang berhubungan dengan metabolisme.

Kata kunci: obesitas, keturunan, penyakit metabolik

INTRODUCTION

Obesity is metabolic disease associated with significant morbidity and mortality, affecting both adults and children. Base of this disease is an energy imbalance, with energy income much higher than energy outcome. This imbalance can be caused by lifestyle and environment, but also by genetic defection in fat metabolism. Obesity is spread worldwide, regarded as a global epidemic. As this disease is spreading among population and getting more and more serious, better understanding of fat metabolism is needed.

Definition and measurements of obesity

Overweight and obesity are both names for ranges of weight that are greater than what is generally considered healthy for a given height. The definition of obesity varies, but in general it means a chronic condition defined by an excess amount of body fat. A certain amount of body fat is necessary for storing energy, heat insulation, shock absorption, and other functions. The normal amount of body fat (expressed as percentage of body fat) is between 25-30 percent in women and 18-23 percent in men. Women with over 33 percent body fat and men with over 25 percent body fat are considered obesed.[1]

Obesity, in absolute terms, is an increase of body adipose tissue mass. As this is difficult to measure, obesity is typically assessed by BMI (body mass index), simple and widely used method.

BMI is calculated by dividing the subject's weight by the square of his/her height. Where *m* is the weight of the subject in kilograms and *h* is the subject's height in metres.

The most commonly used definitions, established by the WHO[2] (World health organization) in 1997, define the following:

Table 1
Classification of Obesity

BMI [kg /m^2]		Obesity class
From	To	
< 18,5	18,5	Underweight
18,5	24,9	Normal weight
25,0	29,9	Overweight
30,0	34,9	Class I obesity
35,0	39,9	Class II obesity
40,0	> 40,0	Class III obesity - Extreme obesity

Source: Vogel (2000)

This table (Table 1) is valid for both adult men and adult women of all ages, but not for children and teenagers. Measuring overweight and obesity in children aged 5 to 14 years is difficult because there is not a standard definition of childhood obesity which could be applied worldwide. For children and teens, BMI ranges above a normal weight have different labels. Additionally, BMI ranges for children and teens are defined so that they take into account normal differences in body fat between boys and girls and differences in body fat at various ages.[3]

Although BMI correlates with the amount of body fat, it is not it´s direct measure. Some people, such as athletes, may have a BMI that identifies them as overweight even though they do not have excess body fat. There are also differences observed between morbidity and BMI in various ethnic populations.[2,3]

Other methods of estimating body fat include measurements of skin-fold thickness and waist circumference, calculation of waist-to-hip circumference ratios, and non-invasive techniques like ultrasound, computed tomography, hydrostatic weighting, bioelectrical impedance analysis, dual energy X-ray absorptiometry and magnetic resonance imaging (MRI).[3]

Epidemiology

The global nature of the obesity epidemic was recognized eleven years ago by the World Health Organization in the United States, where is situation around obesity the worst. For men in USA, 59 percent are overweight, while 20 percent of the total male population is obese. Fewer women are overweight (51%) but more are obese (25%).[4] WHO also expects that by 2015, approximately 2.3 billion adults will be overweight and more than 700 million will be obese. European countries are rapidly approaching the same level as in USA. Among countries in EU, most obese people live in Germany, UK following. The best situation is in France and Italy.[2]

Obesity is not problem only for high-income countries. Rate of obesity is rising worldwide. The rate of obesity is also seen to increase with age at least up to 50 or 60 years old.

Contributing factors

There are several factors contributing to obesity, such as environment, genetics, drugs, and diseases.

People may have obesity because of environment influences, such as workplaces, homes, and communities. When people eat some food, actually they put some calories to the body, which come from carbohydrates, fats, sugars, or proteins. A *calorie* is defined as a unit of energy supplied by food,[5] regardless of its source. Calories consumed by our body have to be balanced by the daily physical activity. But our physical activity is very low, as we no longer need to run away from predators or gain food by manual work. Food is more calorie-dense, employment sedimentary, transport easy. Because of this reasons, energy flow is unbalanced and causes obesity.

Do genes and heredity affect obesity? Research shows that obesity may be caused

also by genetics factors. Estimates for the genetic basis of phenotypic variations in obesity range from approximately 40 to 70 percent.[6] Genetic predisposition is probably the key factor contributing to obesity, as demonstrated by familial aggregation, twin and adoption studies.[4,6]

Genes can directly cause obesity in disorder, such as Bardet-Biedl syndrome and Prader-Willi syndrome.[5] Children born to overweight parents have a greater chance to be obese. This condition occurs with several possibilities, as children can inherit bad habit appetite from their parents, or they inherited genetic predisposition to obesity.

Despite obesity having strong genetic determination, the genetic composition of the population does not change rapidly. Therefore, the large increase in number of obese people must reflect major changes in non-genetic factors.[7]

Obesity may be caused also by some illnesses, for example Cushing's disease, or polycystic ovary syndrome. Drugs such as steroids and some antidepressants may also cause weight gain.[4]

Effect on health

Obesity cause wide range of difficulties. While there are obvious cosmetic and quality-of-life issues related to obesity, the primary focus is on increased mortality and morbidity. Mortality risk varies with BMI. The lower risk is at BMI 22-24 kg/m^2 and increases with changes in both directions. Obesity on average reduces ones life expectancy by 6-7 years. For subjects with severe obesity (BMIs >40) life expectancy is reduced by 20 years for men and 5 years for women.[8]

Clinical problems range from mechanical (osteoarthritis and sleep apnoea) to metabolic perturbations resulting in type 2 diabetes, hyperlipidaemia, hypertension, and overall increased cardiovascular risk.[9] Obesity can also cause difficulties in pregnancy, even a defect of newborn child. In addition, there is an increasing realisation that obesity is strongly associated with a higher risk of developing cancer, for example endometrial, colon and prostate cancer.[9]

Possible treatments and therapies

An overweight patient, doctors and nutritionists must realize that successful treatment and therapies require a lifelong effort. There are several therapies used in obesity treatment:

Dietary Therapy: Dietary therapy means the limitation of calorie income with several terms, conditions, and modifications. The Diets Weight loss therapy is recommended for patients with a BMI ≥ 30 and for patients with a BMI between 25 and 29.9 or a high-risk waist circumference, and two or more risk factors.[10]

Physical Activity: Physical activity plays an important role in weight reduction because it may increase the energy expenditure. This treatment may also reduce the consequences of obesity, such as heart disease.

Behaviour Therapy: Behaviour therapy is an important and difficult part of the obesity treatment because the overweight patient has to invest great effort in doing this treatment. Specific behavioural strategies include the following: self-monitoring, stress management, stimulus control, problem-solving, contingency management, cognitive restructuring, and social support.[10]

Pharmacotherapy: Pharmacotherapy may be helpful for eligible high-risk patients[10] We have to be aware of the chemical compounds of the drug for obesity treatment. The FDA has already approved several drugs for obesity treatment. Currently, NICE (National Institute for Health and Clinical Excellent, in UK) guidelines recommend the use of either orlistat which act as an intestinal lipase inhibitor and effectively induces a partial stage of fat malabsorption, and sibutramine which act in the CNS and inhibits re-uptake of both serotonin and noradrenaline.[9]

Weight Loss Surgery: Surgery is an option for patients with extreme obesity[10] (BMI > 40). Because we do not know yet the exact impact of surgery, patients should be given better knowledge about the risk and complications of the surgery treatment.

DISCUSSION

A base of the problem of the obesity is that the fat metabolism does not work properly. This can be caused by several factors. The problem can be in regulation of appetite and satiety, in the regulation of an adipogenesis or in any other step of the metabolic pathway. As complexity of obesity and human metabolism does not allow its proper investigation in human, we use model

organisms to simplify it and explore in less complex steps. For this purpose four different models are discussed: *Caenorhabditis elegans, Drosophila melanogaster, Danio rerio* and yeast *Saccharomyces cerevisiae*.

Regulation of appetite and satiety

The ability to adjust energy flux in response to changing nutritional status is critical for survival of organism, what is a role of metabolic sensing mechanisms.[6] On a cellular level, metabolic sensors respond to altered concentrations of macronutrients, metabolites derived from these macronutrients and energy resources (ATP, NADH). In multi-cellular organisms, energetic status of different tissues is further coordinated through hormonal signals. Recent studies in mammals indicate that some of these cellular metabolic sensors also function in the nervous system to regulate behavioural responses.[6]

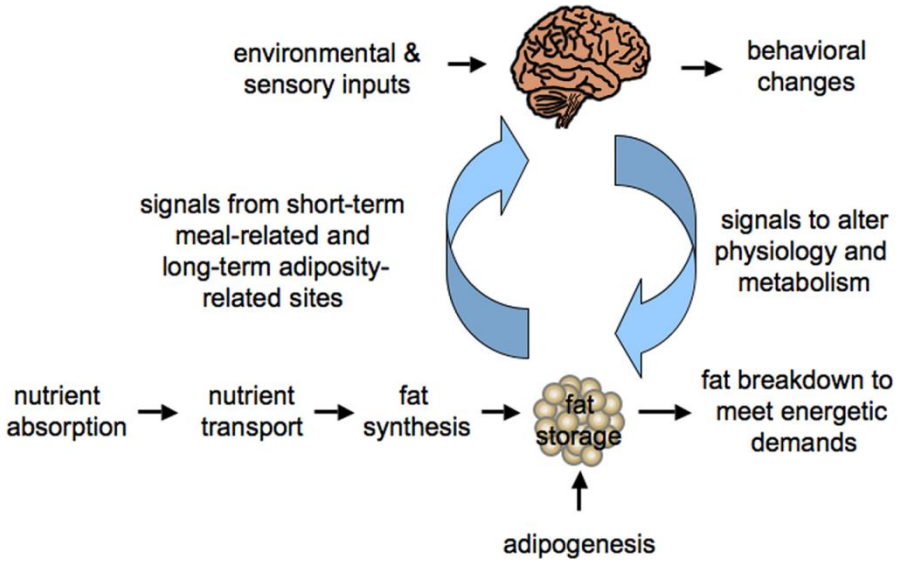

Figure 1. Mammalian homeostatic regulation of energy balance. Signals from fat storage sites report energetic state of the body to the nervous system, which also receives environmental and sensory inputs. The nervous system responds by altering behaviour, physiology, energy uptake and storage.[6]

One of the most important hormones in satiety regulation is leptin. The recognition of leptin deficiency was the basis for obesity observed in the *ob/ob* mouse, en extensively studied rodent model for obesity. Leptin is an adipocyte-derived hormone secreted in white fat tissue as a response to an increase in energy stores. Its concentration in plasma is directly proportional to the degree of body fat. Activation of hypothalamic leptin receptors suppresses food intake and promotes energy expenditure pathways, such as thermogenesis, that creates long-acting effect of reducing adiposity.[9] Another important hormone in control of rodent appetite is melanocortin. Through the identification of number of monogenic disorders, the relevance of the leptin-melonocortin pathway to human appetite regulation was firmly established.[9]

Insulin is another important lipostatic hormone that controls energy balance. Insulin is secreted from the pancreas in proportion to fat mass and trough CNS affects peripheral nutrient storage, causing long-term inhibitory effects on energy intake.

Also some neuro-peptides and neurotransmitters, for example serotonin, dopamin and noradrenalin play role in behavioural, physiological and metabolical responses in relation to mass of fat.[6] Control of appetite is based on highly complex network of interactions and signal from gastrointestinal tract can also impact on appetite control and energy homeostasis.[1] In addition to the long-term adiposity signals, short-term meal-related signals are transmitted to the CNS through afferent nerves or gut-secreted peptides (e.g., cholecystokinin, ghrelin). Finally, neurons in the CNS also directly sense carbohydrate and fats.[6]

Model organism-yeast Saccharomyces cerevisiae

Yeasts are unicellular eucaryotic organisms, and this characteristic makes them an excellent model organisms. As they are eucaryotic, many basic processes of fat metabolism are similar to mammalian. The fact they are unicellular allows us to study the regulation of the lipid metabolism on the molecular level, as well as other evolutionary conserved cellular processes, such as the regulation of the cell cycle.[6]

Storage and degradation of triglycerides are essential processes that ensure energy homeostasis and availability of precursors for membrane lipid synthesis. In mammalian cells, invertebrates, as well as in plants and fungi, triglycerides and steryl esters are packaged into lipid droplets, which are "organelles" consisting of neutral fat surrounded by phospholipid monolayer.[11] Kurat et al explored formation and metabolism of these droplets, several other proteins involved in fat metabolism, storage and transport and their homology to mammalian proteins.[11]

Table 2
Genes related to fat metabolism & their function in Saccharomyces cerevisiae[6]

Genes	Note
tgl4	Major adipose triglyceride lipase of Saccharomyces cerevisiae, an ortholog of mammalian adipose triglyceride lipase
tgl1	Remodeling lipid content of nuclear membrane, essential for sporulation
Ntel	Homolog of the mammalian neuropathy target esterase I with intracellular phospholipase B activity
YOR081c, YOR089c	Ability to mobilize lipids *in vivo*
Vps55p	Protein trafficking, homolg of Human Obesity Receptor Gene-related Protein[16]

This organism has also its disadvantage, e.g. there is no inter-tissue communication, as yeast is only unicellular organism.

Model organism – worm Caenorhabditis elegans (C. elegans)

Study of fat in worm *C. elegans* has more advantages in the regulation, accumulation, and storage of fat at the level of the whole organism. Study of this worm by Avery et al gives another fact that the worms' genes can be analogues and known to be critical for mammalian fat storage. This opened up the possibility that the worm model could be exploited to improve human health[12]

Several important genes that involved in worm metabolic fat have been already discovered and examinated.[12,13]

Based on these information regarding the genes which are involved in mechanism of worm fat metabolic, several researchers conduct study of biotechnology known as RNA interference.[12,13] The study focuses on the genes such as SREBP (sterol regulatory element binding protein) and C/EBP (CCAAT/enhancer binding protein) which have function as the transcription factor. Worms missing one or both genes (SREBP and C/EBP) displayed "lipid-depletion phenotype", lacked of fat accumulation, and had reduced expression of lipogenic enzymes.[12] That means that if we block or knock out either one or both genes, we will have worm with lipid-depletion phenotype. That means, they are pale, skinny and larval-arrested. These worms also lacked fat stores and had reduced expression of lipogenic enzymes, suggesting that the function of SREBP and C/EBP is conserved from nematodes to mammals.[12]

Table 3
Genes related to fat metabolism and their function in C. elegans[12 13]

Genes	Note
glo-1	Required for intestinal lysosomes acidification, mutation of this gene decreases gut granule lipid accumulation.
glo-4	Required for exchanging GDP to GTP for Glo-1, mutation of this gene decreases gut granule lipid accumulation.
glo-5 (pgp-2)	Required for gut granule acidification, links gut granule biogenesis to neutral lipid storage, mutation of this gene affect suppression the glo phenotype.
tub-1	Expressed in the central nervous system and have a role as a transcription factor, mutation of this genes decreased in gut granule lipid accumulation.
kat-1	Localized in mitochondria or peroxisomes, mutation of this gene suppressed the tub-1 mutant.
lpd-3	A novel protein with unknown function. Mutation of this gene especially depletion of this gene caused decreased gut granule lipid accumulation.
rbg-3	Interact with tub-1 with a Rab GTPase activating protein in ciliated neurons.
Scd	Sterol-coA desaturase which is suppressed strongly while fasting.
nhr-49	Expression of this gene encodes nuclear hormone for induction beta oxidation. Mutation of this gene especially depletion elevated fat content and life span.

Many of the health effects of excess fat accumulation in humans are unlikely to occur in *C. Elegans*, but there are many remarkable similarities between molecular components of mammalian and *C. elegans* fat pathways that extend to disease-associated genes. Many of the fat genes identified in *C. elegans* have mammalian homologs whose roles in energy balance have not yet been examined. As the energy balance is fundamental for survival, it is likely that many of the newly identified *C. elegans* fat regulatory networks are functionally conserved in mammals.[6]

Model organism – fruit-fly Drosophila Melanogaster (D. melanogaster)

The use of *D. melanogaster* in obesity research identifies genes responsible for obesity control, body weight regulation, and fat storage. The following Table 4 shows the several important genes involved in flies' fat metabolism.[13]

Several studies have been done with genes in *Drosophila* for manipulating fat regulation. Specific inhibition of Hh signaling in adult mesodermal stem or progenitor cells might be useful treatments for inherited diseases marked by adipose tissue loss and TAG redistribution, the specificity of Hh signalling could be useful for treatment obesity.[13] The processes of knockdown several genes will affects of oenocytes which have function in the capacity of ketogenic hepatocytes. The other factors are protein catabolism and mobilization of amino acids for ketogenesis also results in oenocytes lipid accumulation.[3] Knockdown of fat body cell lsd-2 will decrease oenocyte lipid accumulation; meanwhile overexpression of *bmm* will promote oenocyte lipid accumulation. Summary, the oenocyte lipid accumulation is the important target and signals in fat accumulation in the whole body of *D. melanogaster*.[13]

Through exploration and understanding the function and signalling emanating the genes, especially the oenocytes, we can conduct further research into energy hemeostatis, reproduction, life span, and immune system.

Table 4
Genes Related to Fat Metabolism & Their Function in D. Melanogaster[13]

Genes	Note
Adp (adipose)	Encodes a WD40/tetratricopeptide-repeat-domain protein whose function in regulating TAG accumulation in fat body cells, mutation of this gene increases fat body size and female infertility.
Hh (hedgehog)	A groups of cholesterol-modified proteins involved in multiple development processes, mutation of this gene inhibit fat body development and lipid accumulation.
AKH (adipokinetik hormone)	A neuropeptide that functions like glucagon and β-adrenergic agonists do in vertebrates in counteracting insulin action, mutation of this gene leads to decreased mobilization of fat body lipids.
Lsd-2 (lipid storage droplet-2)	Expressed in the ovary and the fat body, mutation of this genes result in defects in oogenesis, decreased fat body TAG accumulation, and shortened life span.
Bmm (brummer)	Related with TAG mobilization and have the similarity between mammalian and *Drosophila*, mutation of this gene increased fat body lipid contents and sensitive to starvation.

Model organism – zebrafish Danio rerio

Because it is a vertebrate, the zebrafish is genetically closer to humans than flies or worms, and its small size, quick generation time, and inexpensive care make it possible to keep thousands of fish in a single lab.[14] Another advantage of this animal is its transparency when it is young. Since zebrafish eggs are fertilized externally, development and onset of their metabolic activity can be monitored in real time *in vivo*.[3]

One of the zebrafish mutants used as model for obesity is called *jumbo*. This mutant was created by team of The Rockefeller University in New York City, as they try to find mutant fish with similar behaviour as mice missing leptin – with continuos feeding without reaching satiety. They created mutants by exposing zebrafish males to a chemical that causes mutations in sperm-producing cells, and observed their feeding behaviour, by using coloured food and trasparency of fish bodies. The Rockefeller team has identified three families of fish that seem insatiable. *Jumbo* mutant grows considerably larger than normal zebrafish, but another, called *fressack*, is no larger than normal despite its gluttony.[14]

Another type of zebrafish mutant, *gonzo* mutant, has mutation in gene encoding the site 1 protease responsible for processing the ER membrane-tethered transcription factor SREBP, the main regulator of de novo fatty acid and cholesterol synthesis. *Gonzo* mutants are incapable of responding to low cellular cholesterol by activating SHREB, and are thus genetically starved of lipids.[13]

The machinery of lipid synthesis and transport used by humans is presend and active also in zebrafish. Beta lipoproteins are central to the trafficking of yolk-supplied, intestinal and hepatic lipids. The packaging of lipids into the beta lipoproteins is role of microsomal triglyceride transfer protein (Mtp), which is present in zebrafish yolk, intestine and liver. Knockout of zebrafish Mtp results in many of the phenotypes seen in severe, lethal human and mice diseases connected with lipids, with failure to transport yolk lipids and die in utero by starvation.[13]

CONCLUSION

Obesity is one of the major problems in developed countries. Our understanding of fats metabolism and satiety control is crucial for solving this problem. Understanding fat regulation requires multiple layers of investigation, from metabolism, transcription and signaling to neuronal development and behaviour.

Using model organisms help us to understand complex problem of fat metabolism and regulation in pieces which are easier to explore and understand. For this purpose four

different models were discussed: *Caenorhabditis elegans, Drosophila melanogaster, Danio rerio* and yeast *Saccharomyces cerevisiae*.

Every model organism has its own advantages and disadvantages, and we need to understand its relation to mammals. Genes identified by several methods in four models should be examined more properly and we should search for their mammalian homologs. Future work with these models for human therapies must exploit their individual advantages, strengths, and avoid the limitations.

In a future, it would be interesting to focus on.

- Examination of mammalian homologs of genes responsible for fat storage and matabolism in model organisms.
- Identification of genetic polymorphisms underlying naturally-occurring variation in obesity-related phenotypes.
- Studies to identify genes that effect changes in metabolism.
- Identification of genes that are related with both obesity and relating diseases, such as type 2 diabetes, or cardiovascular diseases.
- Development and implementation of screens to study control of eating behaviour, level of physical activity, and metabolic rate.

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REFERENCES

1. WeightCom. *Definition of Obesity*. http://www.weight.com/definition.asp?page=3. Accessed September 2009.
2. WHO. *Preventing and Managing the Global Epidemic of Obesity. Report of the World Health Organization Consultation of Obesity*. Geneva: WHO, 1997.
3. CDC. *Obesity*. http://www.cdc.gov/nccdphp/dnpa/obesity/defining.htm. Accessed September 2008.
4. eMedicine. *Obesity*. http://www.emedicine.com/med/TOPIC1653.HTM. Accessed September 2009
5. National Institute of Health, National Heart, Lung, and Blood Institute~~s~~. *Clinical Guidelines on the Identification, Evaluation, and Treatment of Overweight and Obesity in Adults*: The Evidence Report~~s~~. Washington: National Institute of Health, 1998.
6. Obesity and the regulation of fat metabolism. 2007. *WormBook*, ed. The *C. elegans* Research Community, WormBook, http://www.wormbook.org. Accessed September 2008.
7. Spiegel. 2007. *Overweight and Obesity in 25 EU States.* http://www.spiegel.de/international/europe/0,1518,grossbild-850885-478303,00.html.
8. Vogel G. Zebrafish Earns its Stripes in Genetic Screens. *Science* 2000, 288(5469): 1160-61.
9. Crowley VE~~F~~. Overview of human obesity and central mechanisms regulating energy homeostasis. *Ann Clinical Biochem* 2008; 45(Pt 3): 245-55.
10. Foubister V. *Nematode model for obesity*. *Targets* 2003, 2(2): 40.
11. Kurat CF, Natter K, Petschnigg J, Wolinski H, Scheuringer K, Scholz H, et al. Obese yeast: Triglyceride lipolysis is functionally conserved from mammals to yeast. *The Journal of Biologic~~al~~ Chemi~~stry~~* 2006; 281: 491-500.
12. Hill JO, Trowbridge FL. Childhood obesity: future directions and research priorities. *Pediatrics*. 1998; 571(Supplement): citated in (4)
13. Schlegel A, Stainier DYR. Lessons from "Lower" Organisms: What Worms, Flies and Zebrafish Can Teach Us about Human Energy Metabolism. PloS *Genetics* 2007; 3: e199.
14. Belgereth-Touze N, Avaro S, Rouille Y, Hoflack B, Haduenauer-Tsapis R. Yeast Vps55p, a Functional Homolog of Human Obesity Receptor Gene-related Protein, Is Involved in Late Endosome to Vacuole Trafficking. *Molecular Biology of the Cell* 2002; 13: 1694-1708.