Review Article

บทบาทของภาวะเครียดออกซิเดชั่นและระบบต้านอนุมูลอิสระต่อกลุ่มโรคเมแทบอลิก

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บทคัดย่อ

บทบาทของภาวะเครียดออกซิเดชั่นและระบบต้านอนุมูลอิสระต่อกลุ่มโรคเมแทบอลิก

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ว. เภสัชศาสตร์อีสาน 2556; 9(3) : 1-16

Received : 26 August 2012 **Accepted :** 15 October 2013

อนุมูลอิสระ (reactive oxygen species) เป็นผลิตภัณฑ์จากกระบวนการสันดาปของเซลล์ในร่างกายที่มีความสำคัญต่อระบบต่างๆ ทางสรีรวิทยา อย่างไรก็ตาม สารดังกล่าวสามารถก่อให้เกิดอันตรายจากภาวะเครียดออกซิเดชั่นได้หากขาดความสมดุลระหว่างการผลิตอนุมูลอิสระและขีดความสามารถในการกำจัดอนุมูลอิสระของร่างกาย ภาวะเครียดออกซิเดชั่นส่งผลรบกวนระบบต่างๆ ในร่างกายและเป็นปัจจัยสำคัญหนึ่งของโรคเมแทบอลิกต่างๆ อาทิ โรคอ้วน ภาวะดื้อต่ออินซูลิน และภาวะไขมันพอกตับ เป็นต้น การศึกษาที่ผ่านมาพบความสัมพันธ์ระหว่างการเพิ่มขึ้นของค่าดัชนีมวลกายและเส้นรอบเอวในโรคอ้วนกับการเพิ่มขึ้นของดัชนีบ่งชี้ภาวะลิปิดเปอร์ออกซิเดชั่น ซึ่งได้แก่ กรดไทโอบาร์บิทูริก รีแอคทีฟ สับสแตนซ์ (thiobarbituric acid reactive substances; TBARS) ในพลาสม่า และ 8 เอพิ โพรสตาแกลนดิน เอฟ 2 แอลฟา (8-epi-prostaglandin-F2α) ในปัสสาวะ ร่วมกับการลดลงของระดับอะดิโพเนกติน (adiponectin) และสมรรถนะของเอนไซม์ต้านภาวะเครียดออกซิเดชั่นต่างๆ ได้แก่ ซุปเปอร์ออกไซด์ดิสมิวเทส (superoxide dismutase) กลูตาไธโอนเพอร์ออกซิเดส (glutathione peroxidase) และ คะตะเลส (catalase; CAT) ซึ่งสอดคล้องกับการเพิ่มขึ้นของระดับ TBARS และไฮโดรเจนเปอร์ออกไซด์ในพลาสม่าและเนื้อเยื่อไขมันขาวของหนูไมซ์ที่ถูกชักนำภาวะอ้วนและภาวะเบาหวาน ร่วมกับการลดลงของการแสดงออกของยีนอะดิโพเนกติน และเพอร์ออกซิโซมโพรลิฟเฟอเรเตอร์แอคติเวทรีเซพเตอร์แกมม่า (PPAR-γ) ในขณะที่การแสดงออกของยีนทูเมอร์เนโครซิสแฟคเตอร์อัลฟ่า (TNF-α) พลาสมิโนเจนแอคติเวเตอร์อินฮิบิทเตอร์วัน (PAI-1) และนิโคตินาไมด์อดีนีนไดนิวคลีโอไทด์ฟอสเฟตออกซิเดสสับยูนิต (NADPH oxidase subunits) ต่างๆ กลับเพิ่มขึ้น ภาวะเครียดออกซิเดชั่นในโรคเบาหวานมีความเกี่ยวข้องกับภาวะน้ำตาลในเลือดสูงและภาวะดื้อต่ออินซูลิน โดยส่งผลเพิ่มการสร้าง ROS และ RNS ผ่านกระบวนการการหายใจของไมโตคอนเดรีย (mitochondrial respiration) และวิถีของไนตริกออกไซด์ (nitric oxide pathway) และการรบกวนการแสดงออกของยีนแพนเคลียสดูโอดินัม โฮมิโอบอกซ์ 1 (pancreas duodenum homeobox-1; PDX-1) โดย ROS นั้นสามารถส่งผลต่อการสร้างอินซูลินในโรคเบาหวาน สอดคล้องกับรายงานการเพิ่มขึ้นของระดับคอนจูเกตเทดไดอีน (conjugated dienes) และมาลอนไดอัลดีไฮด์ (malondialdehyde) ซึ่งเป็นดัชนีบ่งชี้ภาวะลิปิดเปอร์ออกซิเดชั่นร่วมกับการลดลงของกลูตาไธโอนและสมรรถนะของเอนไซม์ CAT ในผู้ป่วยโรคไขมันพอกตับ ดังนั้น จึงอาจกล่าวได้ว่าโรคเมแทบอลิกต่างๆ นั้นมีความเกี่ยวพันกับความไม่สมดุลของระบบต้านออกซิเดชั่น หรืออีกนัยหนึ่งการเพิ่มขึ้นของภาวะเครียดออกซิเดชั่นเป็นปัจจัยกระตุ้นหนึ่งของโรคเมแทบอลิก การศึกษาต่อยอดเพื่อความเข้าใจตัวแปรต่างๆ ที่เกี่ยวข้องกับภาวะเครียดออกซิเดชั่นและกลไกของโรคเมแทบอลิก จึงมีความน่าสนใจและจำเป็นต่อการป้องกันหรือชะลอโรคเมแทบอลิกได้

คำสำคัญ: ระบบต้านอนุมูลอิสระ, ภาวะเครียดออกซิเดชั่น, โรคอ้วน, โรคเบาหวาน, โรคไขมันพอกตับ

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Abstract

The Role of Oxidative Stress and Anti-oxidation System in Metabolic Syndrome

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IJPS, 2013; 9(3) : 1-16

Reactive oxygen species (ROS) are cellular metabolic products found in the living body. These species play an essential role in physiological system. However, the deterioration effects under the oxidative stress possibly occur whether imbalance between production of ROS and scavenging capacity is presented. Oxidative stress interferes several biological systems and becomes one important factor in metabolic syndrome such as obesity, diabetes mellitus, and nonalcoholic fatty liver (NAFLD). The linear relationship between increasing body mass index (BMI) and waist circumference and the elevation of lipid peroxidation markers including plasma thiobarbituric acid reactive substances (TBARS) and urine 8-epi-prostaglandin-F2a, with lowering the levels of adiponectin and enzymatic antioxidants, *i.e.*, superoxide dismutase, glutathione peroxidase, and catalase (CAT), has been reported. Correspondingly, levels of TBARS and hydrogen peroxide in plasma and white adipose tissue were increased together with decline of expression of adiponectin and peroxisome proliferator activated receptor gamma (PPAR-g) in the obese and diabetes mice. The expression of tumor necrosis factor alpha (TNF-a), plasminogen activator inhibitor-1 (PAI-1), and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH oxidase) subunits were up-regulated. In DM, oxidative stress was observed involved with prolong exposure to glucose which lead to more generating of ROS and RNS through mitochondrial respiration and NOS pathway. The defection of posttranscriptional in pancreas duodenum homeobox-1 (PDX-1) mRNA maturation by ROS was also observed as a cause to reduced insulin production in DM. Furthermore, correlation between elevation of conjugated dienes and malondialdehyde, the index of lipid peroxidation, and attenuation of glutathione and activity of CAT was shown in the patients with NAFLD. Hence, the metabolic syndrome is associated with disequilibrium of anti-oxidation system. The future studies for understanding a variable influencing oxidative stress and pathogenesis of metabolic syndrome are worth interesting for protection and delay progression of metabolic syndrome.

Keywords: anti-oxidation system, oxidative stress, obesity, diabetes mellitus, nonalcoholic fatty liver.

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Introduction

Reactive oxygen species (ROS) are cellular metabolic products found in the living body. These species play a role in biological response or signaling of neurotransmission. Oxidative stress is a process occurred when cells are pushing to be over production of the free radicals and finally cause damaging. Therefore, the living mechanism has antioxidants to detoxify those harmful radicals for protecting cells (Rolo *et al.*, 2012). The anti-oxidation consists of enzymatic and non-enzymatic systems including superoxide dismutase (SOD), which is the first line anti-oxidation enzyme to catalyze oxygen free radical into hydrogen peroxide (H_2O_2), and then H_2O_2 will be changed to H_2O by catalase (CAT) and/or glutathione peroxidase (GPx). The GPx requires reduced glutathione (GSH) as the electron donor and changes the GSH into oxidized glutathione (GSSG) (Valko *et al.*, 2007). Oxidative stress involves in various diseases including metabolic syndrome such as obesity, diabetes mellitus, fatty liver disease, and others. Incidence of these clusters of metabolic condition is coming up to a health problem worldwide (Haffner, 2006). However, etiologies of these diseases are not clearly understood, while the theories of pathogenesis of metabolic syndrome implicated with oxidative stress have been proposed and widely accepted (Amirkhizi *et al.*, 2012; Kumar *et al.*, 2013; Rolo *et al.*, 2012). Therefore, this review aims to describe the involvement of oxidative stress in the metabolic diseases including the general concept of ROS, oxidative stress, and antioxidant. The roles of ROS in pathogenesis of metabolic diseases are also provided.

Overview of reactive oxygen species (ROS) and oxidative stress

Term of the free radical can be described as a molecule which contains one or more unpaired electrons in its molecule. In physiological event, reactive oxygen species (ROS) are generated to regulate the physiological function in living cell. ROS play an important role in various biological responses such as intracellular killing of bacteria by phagocytic cells, *i.e*., granulocytes and macrophages and are essential signaling molecules in normal physiology. Another physiological function is to modulate transcription of genes such as nuclear factor-kB (Buetler *et al.*, 2004; Valko *et al.*, 2007). However, they also found the involvement of ROS in several diseases including metabolic syndrome, *i.e.,* diabetes mellitus, obesity, non-alcoholic fatty liver, and the aging process (Amirkhizi *et al.*, 2012; Furukawa *et al.*, 2004; Maritim *et al.*, 2003; Narasimhan *et al.*, 2010). In living cell, radicals from the molecular oxygen are the most important class of radical species. The reactive oxygen species (ROS) are reactive molecules containing oxygen with unpaired electron(s) including superoxide radical ($O_2^{\bullet-}$) and hydroxyl radical ($^{\bullet}OH$) (Singh *et al.*, 2009). The formation pathways of ROS consist of many steps (Fig. 1). Superoxide radicals are generated from normal metabolic process of oxygen as a by-product in the living cell. They are considered as the primary ROS which could be followed by formation of the secondary ROS known as hydroxyl radicals. $O_2^{\bullet-}$ is normally generated in body by three main pathways consisted of NAD(P)H oxidase for bacterial destruction in the phagocytic cells, xanthine oxidase (XO), and redox-reactive compounds of mitochondrial electron transport chain (ETC). In the energy transduction processes of ETC for generating electrochemical gradient, some of electrons can leak and form $O_2^{\bullet-}$ with the oxygen molecule. Therefore, the production of $O_2^{\bullet-}$ are mostly found within mitochondria organelle of cell. Then, SOD catalyzes dismutation of $O_2^{\bullet-}$ to H_2O_2, which then being converted into H_2O by others antioxidant enzymes (Dalle-Donne *et al.*, 2006; Rolo *et al.*, 2012; Valko *et al.*, 2007).

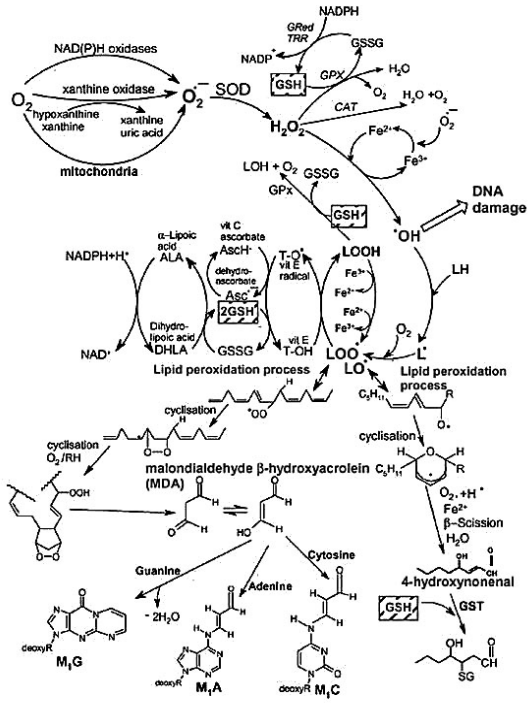

Figure 1 Pathway of ROS formation (modified from Valko *et al.*, 2007)

$^{\cdot}OH$ is another ROS which has the most reactive characters (Dröge, 2008). Some transition metal ions such as Fe^{2+} and Cu^{2+} can changed H_2O_2 to $^{\cdot}OH$. This radical is capable of insulting, fragmenting, and mutating any cellular molecule with forceful intensity. The electron from polyunsaturated fatty acid (LH) is withdrawn to $^{\cdot}OH$ and changed to the lipid radical ($L^{\cdot}$) which can follow to be a lipid peroxyl radical ($LOO^{\cdot}$) after interaction with oxygen molecule. Then, $LOO^{\cdot}$ can cause the damaging process of cell membrane called lipid peroxidation, which has several intermediated products such as 4-hydroxynonenol and malondialdehyde (MDA) (Valko *et al.*, 2007). MDA can cause DNA damaging by interaction with DNA base including cytosine, adenine, and guanine (Marnett, 2002).

Antioxidants

Because the proper levels of free radicals are necessary for life, therefore our body has defense mechanisms against oxidative stress with repairing function to the damage that occurs (Fig 1). The defense system for balancing free radical in living is the cooperation of various antioxidants such as the enzymes, namely SOD, CAT, GPx, and glutathione reductase (GR). The non-enzymatic antioxidant including GSH, vitamin C (ascorbic acid), vitamin E (α-tocopherol), and α-lipoic acid also play a role for prevention of the oxidative stress.

SOD is a major enzyme catalyzing ROS into H_2O_2. There are differentiated forms of SOD in mammals and most chordate. Copper zinc superoxide dismutase (CuZn-SOD) found in intracellular while manganese superoxide dismutase (Mn-SOD) found in mitochondria (Sun *et al.*, 1988). The following enzyme which clears up H_2O_2 into water (H_2O) is GPx and/or CAT. GPx is majority located in mitochondria whereas CAT is mostly found in peroxisome and has a responsibility to decompose H_2O_2 in the cytosol (Evans *et al.*, 2002). In glutathione antioxidation system, GSH is the major soluble antioxidant in cytosol, nuclei, and mitochondria that acts as the electron donor to GPx and be turning to the oxidized from of glutathione, GSSG. The system is completed by GR and $NADP^+$/NADPH to turn GSSG backs to GSH. The main protective role of glutathione toward oxidative stress aside from being the cofactor of GPx is participated in amino acid transporter to pass through the plasma membrane (Comhair and Erzurum, 2012; Valko *et al.*, 2007). The lower ratio of GSH/GSSG which cause from high level of GSSG may predict the damaging of the enzymes in the antioxidant system (Dalle-Donne *et al.*, 2006).

In non-enzymatic antioxidation systems, vitamin E, a lipid soluble antioxidant, plays an important role for scavenging those harmful free radicals and converts itself into vitamin E radicals. Then the vitamin

E radicals will be regenerated back to the functional form by vitamin C and GSH. To complete the system, the GSSG and vitamin C radicals then are regenerated by dihydroxy lipoic acid, which will be converted into α-lipoic acid. Decrease of those cellular antioxidants has been found to increase susceptibility to the oxidative stress state (Evans *et al.*, 2002; Traber and Stevens, 2011). Apart from those mention antioxidants, there are a number of molecules which provides antioxidant capacity in the body such as polyphenols, flavonoids, vitamin A, and melatonin. However, these bio-molecules serve the body as modulators of physiological function rather than the antioxidative role (Azzi and Stocker, 2000)

Metabolic syndrome

Metabolic syndrome or also known as syndrome X, is referred to the combination of risk factors for cardiovascular disease and diabetes (Seneff *et al.*, 2011). These factors are associated with abnormal of carbohydrate and lipid metabolism including insulin tolerance, dysglycemia, centrally distributed obesity, resistance hypertension, and dyslipidemia which consist of elevated high-density lipoprotein (HDL) and/or high levels of triglycerides (Alberti *et al.*, 2009). The resistances to the metabolic effects of insulin were presented (Dandona *et al.*, 2005) (Table 1).

Table 1. Classic biological effect of insulin and classic metabolic syndrome based on resistance to the metabolic effects of insulin (modified from Dandona *et al.*, 2005)

	Normal Insulin Action	**Insulin-Resistant State**
Carbohydrates	↓ Hepatic glucose production	Hyperglycemia
	↑ Glucose utilization	Hyperinsulinemia
	↑ Glycogenesis	
Lipids	↓ Lipolysis	↑ Lipolysis
	↓ FFA and glycerol	↑ FFA and glycerol
	↑ Lipogenesis	↑ Hepatic triglyceride and apoB synthesis
	↑ HDL	↓ HDL
	↓ Triglycerides	Hypertriglyceridemia
		↑ LDL
Proteins	↓ Gluconeogenesis	↑ Gluconeogenesis
	↓ Amino acids	↑ Protein catabolism
	↑ Protein synthesis	↓ Protein synthesis
Purines	↑ Uric acid clearance	Hyperuricemia
	↓ Uric acid formation	

Note. FFA, free fatty acid; HDL, high-density lipoprotein, LDL, low-density lipoprotein

The definition and diagnostic criteria of metabolic syndrome is established by many institutes, one is presented by the US National Cholesterol Education Program Adult Treatment Panel (NCEP) III as a guideline which requires at least three of the conditions following abdominal obesity, high triglyceride, low HDL, high blood pressure, and high fasting blood (Grundy *et al.*, 2005) (Table 2). Apart from NCEP ATP III guideline, the concept of cardiovascular and metabolic risk factor clustering also has been codified by the others organizations but all guidelines agree on the following conditions including abdominal obesity, high fasting blood glucose, dyslipidemia, and elevated blood pressure. WHO focuses either insulin resistance or hyperglycemia as the center of metabolic syndrome with additional related metabolic conditions, and the International Diabetes Federation (IDF) using abdominal obesity determined by waist circumference as the required condition (Huang, 2009).

The underlying cause of this syndrome is still un-fully understood. There are debated about the cause whether they are from environment, inflammation, or metabolic condition (*i.e.* insulin resistance). The cause of this syndrome is related to many causes but all of the risks of the syndrome are related to obesity. The theory of gross dietary imbalances such as excessive consuming of highly processed foods, or fructose was developed to explain the progression of the disease (Seneff *et al.*, 2011).

The relationship between oxidative stress and metabolic syndrome was mentioned in various studies (Amirkhizi *et al.*, 2012; Furukawa *et al.*, 2004; Maritim *et al.*, 2003; Narasimhan *et al.*, 2010). Three conditions including obesity, DM, and NAFLD, will be focused in the present review.

Table 2. The individual risk factors of metabolic syndrome (modified from Grundy *et al.*, 2005)

Risk Factor (Any Three for Diagnosis)	Defining Level
Abdominal obesity: waist circumference	$\geq$ 102 cm or 40 inches for male $\geq$ 88 cm or 35 inches for female
Dyslipidemia: triglycerides	$\geq$ 150 mg/dl or 1.7 mmol/L
Dyslipidemia: HDL cholesterol	< 40 mg/dL or 1.03 mmol/L for male < 50 mg/dL or 1.30 mmol/L for female
Blood pressure	$\geq$ 130/85 mmHg or on antihypertensive drug treatment in patient with history of hypertension
Fasting plasma glucose	$\geq$ 110 mg/dl or 6.1 mmol/L or on drug treatment for elevated glucose

Role of oxidative stress in obesity

Overweight and obesity are conditions being proposed as energy balance disorders with excessive body fat. The common way to diagnosis of obesity is using body mass index (BMI; defined as weight in kilogram per height in squared meter) or using waist circumference. The rate of overweight and obesity is currently a problem worldwide and rapidly increase in both industrialized and developing nations because of life style changed in the last two decades by greater food abundance and lower level of physical activity. According to the record data of World Health Organization (WHO) in 2008, 34% men and 35% of women in worldwide were categorized as overweight (BMI > 25 kg/m^2), while 10% of men and 14% of woman presented as obese (BMI > 30 kg/m^2) These prevalence data is increased double compares to the 1980. The highest prevalence of overweight and obesity were observed in the WHO Regions of the Americas from 62% for overweight in both sexes, and 26% for obesity (World Health Organization, 2008a; World Health Organization, 2008b).

Moreover, obesity is an independent risk factor for coronary heart disease (CHD) and type 2 diabetes by leading to insulin resistance and hyperinsulinemia (Adachi *et al.*, 2006). The increasing risks of developing several diseases such as hypertension, dyslipidemia, gallbladder disease, osteoarthritis, stroke and some type of disease are presented when elevated degree of overweight (Dandona *et al.*, 2005). Several studies have shown that marginal to moderate weight gain in adulthood increases the risk of chronic diseases and negatively affects CHD risk status (Haffner, 2006). Although, there have been evidences of the association between overweight and those diseases, the mechanism of overweight on the pathogenesis of the diseases is not well understood. Oxidative stress is one mechanism that has been proposed as an important factor to promote development and progression of the diseases (Farmer, 2008; Furukawa *et al.*, 2006).

In overweight and obesity, the hypothesis of fat accumulation increased oxidative stress *via* up-regulation of NADPH oxidase leaded to raising up ROS production in cells and decreasing antioxidation enzymes was suggested (Fig. 2; Furukawa *et al.*, 2004). The levels of markers of lipid peroxidation process, plasma TBARS and urine 8-epi-prostaglandin-F2α (8-epi-PGF2α), in the obese subjects excluding patients with the history of diabetes, cardiovascular disease, hepatic disease, renal disease, tobacco abuse, or having hormone replacement therapy were investigated. Besides TBARS, 8-epi-PGF2α is a specific marker of lipid peroxidation process which produced directly *in situ* in phospholipids cell membrane. A correlation between the elevating levels of oxidative injury markers and increases of BMI and waist circumferences were observed while the expression of NADPH oxidase in KKay mice was elevated (Fujitaka *et al.*, 2006; Furukawa *et al.*, 2004). Adiponectin is one of adipocytokine secreted from adipocyte, which exerts the beneficial physiological effect such as enhancing of sensitivity of insulin in the peripheral tissues which leads to improvement of glucose homeostasis, reduction of synthesis of tumor necrosis factor-α proinflammation and anti-artherogenic effects (Farmer, 2008). The level of plasma adiponectin inversely correlated with BMI and waist circumference and plasma adiponectin level also presented a significant inversed correlation with plasma TBARS and 8-epi-PGF2α (Furukawa *et al.*, 2004).

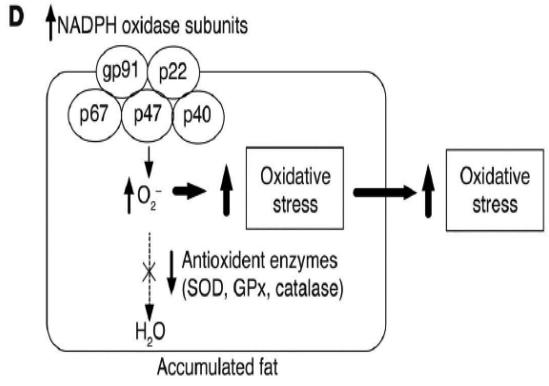

Figure. 2 Increasing production of oxidative stress in accumulated fat model (modified from Furukawa *et al.*, 2004)

Amirkhizi *et al.* (2012) investigated erythrocyte antioxidant enzyme activities including CuZn-SOD, GPx, and CAT in 160 general and abdominal obese women between 20-45 years old, separating to three groups by the body mass index (BMI), namely normal weight (≤ 25 kg/m^2), overweight (25-29.9 kg/m^2), and obese (≥ 30kg/m^2). The decline of main erythrocyte antioxidant enzyme activities in both overweight and obese women wad observed (Table 3). These findings presented the relationship between enzyme activities and obese status after adjusting confounder such as age, physical activity, numbers of pregnancies, blood pressure, and nutrient intakes. Furthermore, a significant association between fat distribution and the level of antioxidant enzymes was found when the subjects were categorized by waist circumference represented the fat distribution. The subjects with abdominal obesity, which is an accumulation of body fat around abdomen, had the lower activities of erythrocyte CuZn-SOD, GPx, and CAT compared to the normal body fat distribution (Amirkhizi *et al.*, 2012) (Table 4).

Table 3. Antioxidant enzyme activities, nutrient intake, and anthropometric characteristics of participants categorized by obesity status (Amirkhizi *et al.*, 2012)

Variables	Normal weight (n = 79)	Overweight (n = 56)	Obese (n = 25)
Weight (kg)	61.3 ± 5.4	71.4 ± 6.1[a]	81.5 ± 7.2[c]
Body mass index (kg/m^2)	23.4 ± 2.2	27.2 ± 2.6[a]	33.6 ± 4.7[b]
Waist circumference (cm)	82.2 ± 3.4	82.8 ± 4.5	83.3 ± 6.6
Waist-to hip ratio	0.74 ± 0.06	0.76 ± 0.05	0.79 ± 0.08
CuZn-SOD (U/gHb)	987 ± 84	721 ± 81[b]	638 ± 74[c]
GPx (U/gHb)	148.7 ± 54.2	124.6 ± 48.9	97.6 ± 45.2[b]
CAT (K/gHb)	316.7 ± 65.8	296.6 ± 48.9	184.2 ± 37.8[b]

Note. [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ from the post hoc comparisons (Tukey test) between overweight or obese subjects vs. normal weight subjects.

In animal model, the increase of oxidative stress in plasma and parametrial white adipose tissue was determined in KKAy mice compared with the age-matched non-diabetic C57BL/6 mice (Furukawa *et al.*, 2004). Due to leptin resistance, the KKAy strain represents the characteristics of genetical obese, hyperphagia, and diabetes, which further develops to severe hyperinsulinemia and hyperglycemia within 3 months. Therefore, the KKAy was used as a type 2 diabetes mellitus model (Adachi *et al.*, 2006; Diani *et al.*, 1987). At age of seventh week and thirteenth week, the KKAy mice developed to obese and diabetes, respectively. Increasing body weight, parametrial white adipose tissue weight, and plasma glucose levels with increasing levels of plasma H_2O_2 and plasma TBARS was found. Furthermore, the levels of TBARS of parametrial white adipose tissues and H_2O_2 production were markedly increased, while no significant differences in other examined tissues, livers, muscle, and aorta, were observed.

In the white adipose tissue, down-regulation of mRNA expressions of adiponectin and peroxisome proliferator activated receptor gamma (PPAR-γ) were shown, while up-regulation of tumor necrosis factor alpha (TNF-α) and plasminogen activator inhibitor-1 (PAI-1) genes were noted. The expressions of NADPH oxidase subunits including $gp91^{phox}$, $p22^{phox}$, $p40^{phox}$, $p47^{phox}$, and $p67^{phox}$, and PU.1, the transcription factor up-regulated the expression of NADPH oxidase, were the most inducible in white adipose tissue (Furukawa *et al.*, 2004).

These results supported the hypothesis of role of oxidative stress in obesity with increasing fat accumulation, resulting in increase of NADPH oxidase subunits mRNA expression with lowering activities of antioxidant enzymes, followed by contributing to ROS production in the accumulated fat.

Table 4. Antioxidant enzyme activities, nutrient intake, and anthropometric characteristics of participants categorized by body fat distribution (modified Amirkhizi *et al.*, 2012)

Variables	Normal body fat distribution (n=98)	Abdominal obesity (n = 62)
Weight (kg)	64.4 ± 6.3	76.4 ± 5.1[b]
Body mass index (kg/m^2)	26.6 ± 3.4	27.4 ± 4.7
Waist circumference (cm)	81.5 ± 5.2	96.3 ± 6.0[a]
Waist-to hip ratio	0.76 ± 0.03	1.02 ± 0.04[b]
CuZn-SOD (U/gHb)	871 ± 84	632 ± 76[c]
GPx (U/gHb)	154.7 ± 62.4	103.6 ± 52.8[b]
CAT (K/gHb)	298.3 ± 71.1	216.6 ± 76.4[a]

Note. [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ from the independent t-test between normal body fat distribution subjects vs. abdominal obesity

Role of oxidative stress in diabetes

Diabetes mellitus (DM) is one metabolic disorder involved with the conditions of hyperglycemia and inadequacy of secretion or action of endogenous insulin (Maritim *et al.,* 2003). The majority of patients suffered from type 2 DM, while only approximately 10% of patients have type 1 (Singh *et al.*, 2009). The character of type 2 DM is a non-insulin dependent type which can produce insulin to stimulate up-taking of glucose. However, the cellular response is deficient to insulin, therefore this type of DM also known as insulin resistance. This condition results in diminish of up-taking of glucose into muscle and adipose tissue, which lead to prolong hyperglycemia and the related-complication (Valko *et al.*, 2007). The incidence of type 2 DM is very high compared to other types with exponentially rising trend. Aside from adult, raising incident in children and adolescence also is the problem (Singh *et al.*, 2009). The diabetes-related complications, *i.e.* vascular system, kidney, retina, peripheral nerve, and skin, are also the serious problems affected human health worldwide (Maritim *et al.,* 2003).

In ROS generation pathway, oxidative stress process is occurred from either increasing production of ROS or weakening of ROS scavenging from decline of antioxidant system. The concept of relationship between elevation of oxidative stress and progression of type 2 DM is widely accepted to be participate in hyperglycemia-induced trigger of diabetic complications (Baynes and Thorpe, 1999; Maritim *et al.,* 2003; Robertson *et al.*, 2003). The significant biomarkers of oxidative stress implicated with DM include MDA, GSH/GSSG ratio, s-glutathionylated protein, F2-isoprostanes, nitrotyrosine (3-NO_2-Tyr), and advanced glycation end products (AGE) (Dalle-Donne *et al.*, 2006). The source of ROS that causing oxidative stress was proposed as hyperglycemia of the organism leading to induction of ROS generation in various sources including oxidative phosphorylation, glucose autooxidation NAD(P)H oxidase, lipooxygenase, cytochrome P450 monooxygenases, and nitric oxide synthase (NOS) (Valko *et al.*, 2007). The most affected processes to enhance ROS production in insulin resistance conditions and diabetes are mitochondrial respiration and nitric oxide synthesis.

Overproduction of ROS in insulin resistance and diabetes with the uncoupling hypothesis is introduced. To generate the energy or ATP to cells, mitochondrial oxidative phosphorylation is conducted with the electron transfer chain through the complex I (NADH-ubiquinone oxidoreductase), complex II (succinate-ubiquinone oxidoreductase), complex III (ubiquinol-cytochrome c reductase), and complex IV (cytochrome *c* oxidase). The leaking of electron during this process causes the generating of $O_2^{\bullet-}$ and rapidly quenches by the influx of protons through ATP synthase (also known as complex V). The persistence hyperglycemia leads to the excess glucose, and a significant increase of electron donors (NADH and FADH). The activity of mitochondrial electron transfer chain results in increasing supply of electrons. However, abundance of enzyme is limited, therefore this system will saturate and leads to the availability of excess electrons that can interact with O_2 and formation of $O_2^{\bullet-}$. For this reason, electron transfer and oxidative phosphorylation are uncoupled and results in $O_2^{\bullet-}$ formation and inefficient ATP synthesis (Mehta *et al.*, 2006). Besides ROS, there are another reactive species, NO•, generally produced in the body which are the nitrogen reactive species (RNS). This radical generates from the metabolism of arginine to citrulline by nitric oxide synthase (NOS). NO• can react with superoxide and form peroxynitrile anion ($ONOO^-$), which is a potent oxidizing agent that causing DNA fragmentation and lipid peroxidation. In hyperglycemia condition, prolonged exposure to high glucose induced endothelial NOS gene expression.

In diabetes, hyperglycemia condition can lead to exhaustion of NADPH, which is the cofactor for GSH/GSSG and the cellular antioxidant. This mechanism

occurs through the polyol pathway by involving with aldose reductase. The function of aldose reductase is to change aldehydes to alcohols. However, in the state of hyperglycemia, glucose is actively reduced to sorbitol, and then, oxidized to fructose, which the large amounts of NADPH are used (Lee *et al.*, 1999).

There is a hypothesis about the action of glucose toxicity in pancreatic β-cell. The condition of hyperglycemia is prolong high concentrations of glucose, leading to increase of intraislet peroxide level including the formation of O•-, H_2O_2, and OH•. Because of low catalyzing capacity of in the islets, those free radicals are still remained. The OH• is a highly toxic radical which easily passes through nuclear membrane and causes DNA mutation. This ability aids OH• to interfere with the normal process of pancreas duodenum homeobox-1 (PDX-1) mRNA which is a necessary transcription factor for insulin gene expression and glucose-induced insulin secretion. Therefore, this action causes decrease of insulin synthesis and secretion (Robertson *et al.*, 2003) (Figure. 3). Due to glucose toxicity of the pancreatic β-cell is considered to play as a secondary role in the pathogenesis of type 2 DM, the related study has been conducted using HIT cell, which is pancreatic islet β-cell line that could produce and secrete insulin, to investigate whether prolong exposure to glucose suppresses the insulin RNA expression. The results showed the expression of the insulin RNA at 0.5 k base pair using 1.1 mM glucose contained media (Figure. 4). The expression of insulin in the glucose contained media was decreased by the higher order of passage, and subsequently being absent at the 131th passage. The higher order of passage indicated the longer duration of the cells exposed to glucose, therefore, these results implied glucose toxicity resulted in the suppression of insulin production (Robertson *et al.,* 1992).

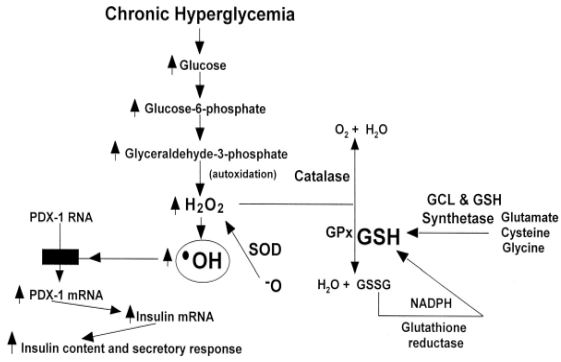

Figure. 3 Mechanism of action of β-cell glucose toxicity (Robertson *et al.*, 2003)

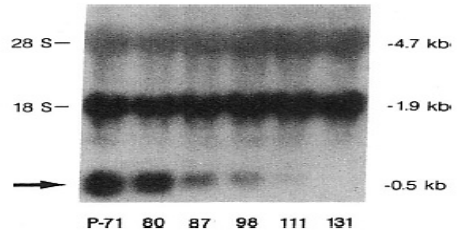

Figure. 4 Levels of insulin mRNA in HIT cell culture using 1.1 mM glucose contained media (Robertson *et al.*, 1992)

The serial passages of HIT-T15 was determined the effect of glucose toxicity to PDX-1 by using nuclear extract of the late passage of cell comparing to the early passage. The disappearance of relative PDX-1 expression in HIT cells containing 11.1 mM glucose media was noted. The decline of PDX-1 gene expression was protected dose dependently by the antioxidant, N-acetylcysteine (NAC). These results supported the hypothesis that the glucose toxicity decreased the expression of PDX-1 and resulting in losing insulin content and insulin secretion.

Role of oxidative stress in nonalcoholic steatosis

Nowadays, the higher incidence of obesity such as diabetes, cardiovascular disease and non-alcoholic fatty liver (NAFLD) leads to various health problems and also considers as the social problem in Western (Lam *et al.*, 2009). NAFLD is recognized as part of metabolic syndrome. This condition defines as the accumulation of lipid, especially, the triglyceride in the liver of a patient who does not take the significant amount of alcohol (< 20 g ethanol/day; ≤ 21 drinks on average per week in men and ≤ 14 drinks on average per week in women) or known causes such as toxins or drugs (Al-Busafi *et al.*, 2012; Zivkovic *et al.*, 2007). The fat accumulation was reported approximately 5-10% by weight of the liver (Rolo *et al.*, 2012). NAFLD can be graded into 4 types based on severity, namely steatosis alone (type 1), steatosis with inflammation (type 2), steatosis with hepatocyte injury or ballooning degeneration (type 3), and steatosis with sinusoidal fibrosis, Mallory bodies, or both two pathologies (type 4) (Zivkovic *et al.*, 2007).

The most severe form of NAFLD included type 3 and type 4 defines as a non-alcoholic steatohepatitis (NASH) (Zivkovic *et al.*, 2007). In North America population, 30% was considered to have NAFLD, and 10% of the patients considered as NASH (Al-Busafi *et al.*, 2012). Although simple steatosis appears to be non-progressive, NASH is possibly further developed to cirrhosis, hepatocellular carcinoma, and liver failure. These adverse clinical outcomes lead to the liver-related death (Rolo *et al.*, 2012; Zivkovic *et al.*, 2007).

The pathogenesis of NAFLD and NASH are still not fully understood but the current concepts of these diseased were related to insulin resistance, obesity, diet and nutrition, lifestyle, and genetic (Zivkovic *et al.*, 2007). There is the hypothesis of the generating of NASH by the “two hit” theory proposed in 1998 (Day and James, 1998) which is the progression from simple fatty liver to NASH. The first hit of the developing this condition is prolong over nutrition for long time and results in accumulation of liver fatty acid and triglycerides from increasing inflow free fatty acids and *de novo* lipogenesis. The second hit of the steatosis is to develop to NASH involved with many factors including the oxidative stress process, mitochondrial dysfunction leading to decrease hepatic ATP production, and trigger necroinflammation by inflammatory cytokines (Rolo *et al.*, 2012).

In the clinical study, the conjugated dienes (CD), the early products, and MDA, the end products of lipid peroxidation in the pathogenesis of NAFLD was significantly increased (Table 5) while the antioxidants, GSH, CAT, were marked reduced (Kumar *et al.*, 2013) (Table 6).

Table 5. Levels of markers of lipid peroxidation in NAFLD patients and healthy volunteers (modified from Kumar *et al.* 2013)

Parameters	Healthy volunteers	NAFLD
MDA (μmol/L)	1.45 ± 0.18[c]	2.41 ± 1.22
CD (μmol/L)	16.45 ± 1.75[c]	24.66 ± 3.23

Note. N = 25; [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ compared between healthy volunteers vs. NAFLD

Table 6. Antioxidant status parameters in NAFLD patients and healthy volunteers (modified from Kumar *et al.* 2013)

Parameters	Healthy volunteers	NAFLD
GSH (μmol/gHb)	3794.48 ± 818.97	2084.53 ± 576.06[b]
GR (units/gHb)	6.88 ± 1.61	7.86 ± 2.01
GPx (units/gHb)	18.17 ± 4.09	25.74 ± 5.19[a]
CAT (μmol H_2O_2 oxidized/min/gHb)	2162.00 ± 198.57	1667.88 ± 192.43[b]
SOD (units/mL)	54.28 ± 9.00	64.90 ± 4.65

Note. N = 25; [a]$p < 0.05$, [b]$p < 0.01$, and [c]$p < 0.001$ compared between healthy volunteers vs. NAFLD

Feillet-Coudray *et al.* (2009) focused on the oxidative stress in wistar rats fed with a high-fat high-sucrose diet for 12 week and found the development of the hepatitis steatosis being recognizable by preponderance of large droplets in which a single, bulky fat vacuole distends the hepatocyte and pushes the nucleus and cytoplasm to the side compared to the control (Figure. 5) and found the dysfunction of mitochondria system in liver while no modification of NADPH oxidase (Feillet-Coudray *et al.*, 2009).

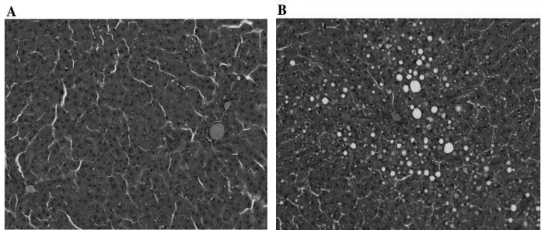

Figure. 5 Liver histology after hematosine/eosine staining in (A) control diet and (B) high-fat high-sucrose diet (Feillet-Coudray *et al.*, 2009)

Conclusion

Metabolic syndrome including obesity, DM, and NAFLD has been found to be implicated with the imbalance of antioxidation system. Increasing of BMI and waist circumference in obesity related to an increase of lipid peroxidation and lowering levels of anti-oxidation enzymes, SOD, GPx, and CAT, and adiponectin. Regarding adipose gene expression, down-regulation of adiponectin and PPAR-γ were noted in the obese and diabetes mice while the expressions of TNF-α, PAI-1, and NADPH oxidase subunits were up-regulated lead to increase of ROS production. In DM patients, oxidative stress was noted by rising of oxidative stress markers such as MDA, F2-isoprostanes, and nitrotrosine with the involvement of hyperglycemia and insulin resistant conditions, leading to increase of ROD and RNS production via mitochondrial respiration and NOS pathway. The role of hydroxyl radicals in glucose toxicity of β-cells was implicated with a posttranscriptional defection in PDX-1 mRNA maturation, which latter suppressed insulin synthesis and secretion. In NAFLD, oxidative stress involved in the pathology of the disease by increasing lipid peroxidation process with increasing the CD and MDA levels and decreasing antioxidants such as GSH and CAT. Therefore, the future studies for understanding a variable influencing oxidative stress and pathogenesis of metabolic syndrome are worth interesting for protection and delay progression of metabolic syndrome.

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