

## 5. DISCUSSION

Type 2 diabetes mellitus is a metabolic disorder of fuel homeostasis characterized by hyperglycemia and altered lipid metabolism caused by islet  $\beta$  cells being unable to secrete adequate insulin in response to varying degrees of over nutrition, inactivity, consequential overweight or obesity, and insulin resistance. The burden of this disorder is enormous, owing to its rapidly increasing global prevalence, the devastating damage it can do to many organs of the body, and the direct and indirect costs. It is commonly accepted that type 2 diabetes results, on the one hand, from population aging and, on the other hand, from adverse environmental factors of the modern world (i.e., high-caloric diets, physical inactivity, and a sedentary lifestyle) which favor the development of obesity. In fact, excess body weight represents a major risk factor for type 2 diabetes<sup>(134)</sup>. Over the last 50 years diabetes is growing rapidly. Although some of the increase in diabetes prevalence may be due to the increasing longevity of the population, an increase in the rate of type 2 diabetes is also being observed among the young, suggesting that an active process is driving the epidemic<sup>(134)</sup>. The estimated worldwide prevalence of diabetes among adults was 285 million (6.4%) in 2010, and this value is predicted to rise to around 439 million (7.7%) by 2030 and Egypt will be ranked as the 10<sup>th</sup> country worldwide which has the highest number of people with diabetes, making it one of the most serious diseases of humankind<sup>(33,134)</sup>. Identifying the etiology and the early detection of type 2 diabetes are keys to prevention. The frequent association of diabetes with obesity has led many investigators to propose that obesity and associated insulin resistance may be responsible for up to 90% of type 2 diabetes<sup>(135,136)</sup>. Since skeletal muscle is a key metabolic tissue, accounting for about 80% of total glucose disposal under insulin-stimulated conditions<sup>(137)</sup>, also adipose tissues and liver play important role in glucose homeostasis, defects of insulin action in these tissues are central to the pathogenesis of type 2 diabetes.

Among the trace elements essential for human health, selenium (Se) stands out for its unique biochemistry, its antioxidant capacity and its narrow therapeutic window. Selenocysteine, the selenium analog of cysteine, is co-translationally incorporated into 25 human selenoproteins. Glutathione peroxidases (GPx), selenoprotein P (SeP) and thioredoxin reductases are the most prominent and ubiquitously expressed selenoproteins, contributing to degradation of reactive oxygen species (ROS) and regulation of cellular redox homeostasis<sup>(36)</sup>. An anti-diabetic impact of dietary selenium supplementation would be expected given both the long track of selenium as insulin – mimetic micronutrient and its antioxidant capacity as constituent of ROS- detoxifying selenoenzymes, suggesting a protective role against oxidative stress – related chronic complications in the progression of diabetes<sup>(36,95)</sup>. Contrarily to those expectations, recent epidemiological and intervention studies revealed a surprising association between high plasma selenium levels and type 2 diabetes, hyperglycemia and dyslipidemia<sup>(38,39)</sup>. The mechanism of the potential diabetogenic effect of excess selenium is not completely understood.

The fortification of foodstuffs with vitamins, minerals and trace elements including Se by fertilization and animal nutrition is widely practiced<sup>(138,139)</sup>. Thus, consumers on the one hand take additional amounts of these substances unknowingly in their daily diet. On the other hand, a wide choice of vitamin and mineral supplements, originally designed to treat and prevent deficiency syndromes, are freely available in supermarkets<sup>(140)</sup>. Consumers frequently misuse these supplements in the belief that these will protect them

from cancer<sup>(141)</sup> and other diseases of civilization or just improve their general health<sup>(140)</sup>. Data from literature have increasingly reported on the inefficacy or even adverse effects of vitamins and trace element supplements on health<sup>(34,142)</sup>. The benefits and risks of Se supplements for the prevention of obesity and Type II diabetes are currently also the subject of controversial discussion. So, this study was designed to explore the suggestion that selenium and selenoproteins may interfere with the insulin secretion from pancreas and/or insulin signaling in peripheral tissues which may cause insulin resistance and subsequently type 2 diabetes and/or obesity.

Tissue levels of Se have long been used as chemical biomarkers to determine Se status and requirements in Se-deficient vs. Se-adequate animals or humans<sup>(143)</sup>. Tissue levels with supranutritional Se supplementation, however, are far less indicative of level of Se exposure because organic and inorganic forms are metabolized differently, incorporated differently into tissue selenoproteins and general proteins<sup>(144)</sup>, and even excreted in different proportions<sup>(145)</sup>. Se-Glutathione peroxidase (GPx-1) is the most abundant biochemical form of body selenium, and its activity could indicate the level of selenium and selenoproteins in organism<sup>(146,147)</sup>. The liver GPx-1 mRNA and activity was used to assess Se requirements in growing rats<sup>(148-150)</sup>, showing that GPx-1 levels can be used as specific biomarkers for nutrient status. Another important biomarker of Se status is selenoprotein P (SeP) which is the main supplier of selenium to tissues in the body<sup>(151,152)</sup>. SeP reaches its maximal expression when serum selenium concentrations are considerably higher than those required to maximize glutathione peroxidase activity.

As expected the results of this experimental study indicated that the Se supplementation cause a dose-dependent elevation in the tissues levels of GPx-1 activity and SeP. However there are differential effects of Se supplementation on these markers of Se status depending on the form of Se (organic or inorganic) and on the type of peripheral tissue. The organic form Se (selenocysteine) showed more prominent effect than inorganic form (sodium selenate) on the activity of Se-GPx (GPx-1) in liver, muscle and adipose tissues. While the same pattern is observed in liver and muscle with respect to SeP level, in adipose tissues this pattern is reversed because the sodium selenate supplementation increase SeP level greater than selenocysteine supplementation. On the other hand the selenocysteine supplementation causes significant inhibition of non-Se dependent GPx (n-Se-GPx) in adipose tissues while liver and muscle showed mild inhibition while sodium selenate supplementation significantly activate n-Se-GPx in muscles at the highest dose (100 µg/Kg) and significantly inhibit its activity in the adipose tissues at doses of 10 and 50 µg/Kg. The data indicated that a used Se compound doesn't have renal toxicity as indicated with normal urea and creatinine levels.

Both inorganic and organic Se are transformed differently into common intermediate; selenide. Inorganic Se (sodium selenate) is reduced simply into selenide while organic Se (selenocysteine) is lysed by β-lyase into selenide. Selenide is endogenously transformed into selenocysteine on tRNA and then selenocysteine residues are incorporated into the selenoproteins by codon specific for selenocysteine, UGA<sup>(153)</sup>. It was documented that the renal excretion of Se after selenate administration is higher than that of organic Se which mean that selenocysteine is accumulated in the tissues which may explain the more efficient effect of selenocysteine on selenoprotein, GPx and SeP especially in liver and muscle.

From the present study it is clear that the long-term supplementation of rats with seleno-compounds result significant dose-dependent increase in fasting blood sugar compared to control (Table 2 and Figure 13). The rats treated with inorganic selenium (Sodium selenate) showed a non-significant higher fasting blood sugar level than the rats treated with organic selenium (selenocysteine). Only the rats treated with the low dose (10 µg/kg) of sodium selenate showed a significant higher fasting blood glucose level than the rats treated with similar dose of selenocysteine. This increase in fasting blood glucose is associated with significant dose-dependent decline in fasting insulin level in the rats treated with sodium selenate while those rats treated with selenocysteine showed no significant change in the insulin level compared to untreated rats (Table 3 and Figure 14).

Many epidemiological studies confirm the link between Se supplementation and diabetes risk. Data from the U.S. National Health and Nutrition Examination Surveys (NHANES) indicate that serum selenium is positively correlated with an increased incidence of type 2 diabetes<sup>(39,154)</sup>. Furthermore, in the French randomized placebo-controlled Supplementation en Vitamines et Minéraux Antioxydants (SU.VI.MAX) trial, selenium was the only antioxidant nutrient tested that was positively associated with increased fasting plasma glucose, which is a precipitating factor in the development of diabetes<sup>(155)</sup>. In addition, another study indicates that individuals that had the highest baseline plasma selenium levels were at an increased risk for type 2 diabetes even when factors, such as age, sex, body mass index and smoking status were controlled<sup>(156)</sup>. Also, high plasma Se was associated with a decreased risk of onset of hyperglycemia over a nine-year follow-up period among male participants in the prospective French Epidemiology of Vascular Ageing (EVA) study<sup>(157)</sup>. The Nutritional Prevention of Cancer (NPC) trial, carried out in the eastern US, showed a significantly increased risk of type-2 diabetes in those supplemented with Se (200 mg/day as Se-yeast) over an average period of 7.7 years<sup>(38)</sup>. Another important prospective study indicated that a 16-year follow-up period of 253 women indicate increased dietary selenium intake was associated with an increased risk of type 2 diabetes<sup>(158)</sup>.

The mechanism(s) of this diabetogenic effect of selenium is still unclear, however some hypotheses have been proposed last years. The normal glucose and insulin homeostasis is based on glucose metabolism in β-cell of pancreas by what is called Glucose-Stimulated Insulin Secretion (GSIS). The glucose sensing apparatus in β-cell is composed of low affinity glucose transporter (GLUT2) and low-affinity glucose phosphorylating enzyme glucokinase<sup>(159)</sup>. The classical scheme of GSIS is depends on the production of ATP molecules by cytoplasmic and mitochondrial metabolism of glucose<sup>(160)</sup>. The ratio of ATP/ADP increases as processing of the glucose increase, leading to closure of  $K_{ATP}$  channels causing depolarization of the plasma membrane and opening of  $Ca^{2+}$  channels. The entry of  $Ca^{2+}$  then triggers exocytosis of the insulin granules. However, the precise signals that couple glucose metabolism to insulin secretion are still incompletely understood. Recently, many suggest that reactive oxygen species (ROS) derived from mitochondrial glucose metabolism are potential metabolic signals that facilitate insulin secretion<sup>(35,161)</sup>. One of the proposed hypotheses to explain the diabetogenic effect of Se suggested that over supplementation with Se may inhibit GSIS from pancreas through over-activation and/or expression of the antioxidant seleno-proteins especially Se-GPx in pancreatic tissues which eliminate the physiological levels of hydrogen peroxide involved in GSIS. In line with this suggestion our correlation data indicated a negative correlation between insulin level and the activity of GPx in the liver.

Selenium may also accumulate in the pancreatic tissue of several animals<sup>(131)</sup>. Also, the histopathological study of pancreas indicated degenerated islet of irregular shape with cytoplasmic vacuolization and apoptotic cells in those treated with high doses of sodium selenate. The decreased insulin level in the sodium-selenate treated rats provides a confirmation of this hypothesis. The rats treated with selenocysteine showed non-significant change in the insulin level compared to untreated control rats however; they showed dose-dependent increase in fasting blood glucose level. These may suggest that that selenocysteine induce diabetogenic phenotype through mechanism not related to insulin secretion but related to insulin action.

The results of this experimental study indicated that the rats treated with the different forms of Se showed a dose-dependent increase in HOMA-insulin resistance index which indicate an insulin resistance state in these rats. Therefore, insulin is unable to act properly on resistant tissues and this resulted in poor glucose disposal, so  $\beta$ -cells initially compensated for insulin resistance by increasing insulin secretion till  $\beta$ -cell exhaustion and apoptosis could occur. Multiple organs contribute to the development of peripheral insulin resistance, with the major contributors being skeletal muscle, liver, and adipose tissue<sup>(162)</sup>. This dose-dependent insulin resistance state was associated with a dose-dependent decline in the phospho-insulin receptor (active fraction of insulin receptor) and glucose transporter 4 (Glut4) in the peripheral tissues especially liver and muscle.

According to these results, the increased fasting blood glucose and insulin resistance with high selenium supplementation might be explained by the effect of high selenium on insulin signaling in peripheral tissues. Binding of insulin to its receptor initiates the insulin signaling cascade, which is accompanied by a burst of hydrogen peroxide that acts as a second messenger.<sup>(163)</sup> High activity of GPx, which removes hydrogen peroxide, might thus interfere with insulin signaling. For example, transgenic mice overexpressing GPx developed insulin resistance, hyperglycaemia, hyperinsulinaemia, and obesity,<sup>(104)</sup> and a strong correlation was noted between increased erythrocyte GPx activity and mild insulin resistance in pregnant women<sup>(164)</sup>. By contrast, knockout of GPx improved insulin-induced glucose uptake and insulin resistance in mice<sup>(163)</sup>. Also, it was documented that two selenium compounds, sodium selenite and methylseleninic acid counteract insulin-induced signaling in myocytes cell line<sup>(165)</sup>. In line with these studies our study indicated a positive correlation between fasting blood glucose level and Se-GPx in the liver, muscle and adipose tissues of the rats supplemented with the different forms of Se.

In previous supplementation studies using differentiated rat L8 myocytes, addition of several forms of selenium (selenite, selenomethionine, and selenocysteine) to the culture medium has been demonstrated to stimulate a dose- and time-dependent up regulation of Selenoprotein W (SeW) and GPx<sup>(166,167)</sup>. The supplementation of rat L6 myotubes with four different Se compounds at doses of 1  $\mu$ M, sufficient for robust induction of SeW and GPx<sup>(166)</sup>. It was observed that a greater variation in the ability of the applied Se compounds to induce an increase in steady-state mRNA levels of SeW and GPx than in their protein expression levels. This discrepancy might be related to the many levels of control implicated in the Se-regulated biosynthesis of selenoproteins, as it is conceivable that Se compounds may act unequally on gene transcription, mRNA stability, translation and posttranscriptional mechanisms<sup>(165)</sup>. Similarly, in a large supplementation comparison study of 10 Se compounds in HepG2 hepatoma cells, GPx and SeP were induced

efficiently<sup>(168)</sup>. Hirofumi Misu et al<sup>(169)</sup> reveals that hepatic overproduction of SeP contributes to the development of insulin resistance in the liver and skeletal muscle, and this may also be the reason of inducing diabetes mellitus.

However, GPx cannot be the only relevant selenoprotein because plasma GPx activity is maximized well below the selenium doses associated with increased risk of type-2 diabetes. Indeed, individuals with a reduced ability to synthesize many selenoproteins had enhanced systemic and cellular insulin sensitivity<sup>(170)</sup>. Another selenoprotein implicated in diabetes risk is SeP, which requires a higher selenium intake than does GPx to achieve maximum plasma concentration<sup>(118)</sup>. SeP functions as a negative insulin modulator: it inhibits the insulin-induced burst of reactive oxygen species *in vitro* and further contributes to insulin resistance by inactivating adenosine monophosphate (AMP)-activated protein kinase (AMPK)—a positive regulator of insulin synthesis and secretion in pancreatic islet  $\beta$  cells<sup>(171)</sup>. Indeed, selenoprotein P has been recently demonstrated to induce insulin resistance in hepatocytes and myocytes. The present study provides further support of the role of SeP in inducing diabetic phenotype especially in the rats supplemented with selenocysteine, as its level in liver, muscle and adipose tissues are strongly positively correlated with fasting blood glucose, also its level in muscle is negatively correlated with Glut4 level, however these correlations are absent in the rats supplemented with sodium selenate. In accordance with our data, clinical studies in Japanese adults have shown that serum SeP concentration is significantly correlated with glycated hemoglobin A1c (HbA1c) and fasting plasma glucose, and is raised in people with type-2 diabetes. Furthermore, serum SeP concentrations were significantly higher in Korean patients with type-2 diabetes or pre-diabetes than in those with normal glucose tolerance and decreased in a stepwise manner<sup>(171)</sup>. SeP was higher in overweight and obese participants than in lean participants, indicating that dysregulated carbohydrate metabolism could be driving the increase in SeP through the action of PGC1 $\alpha$ —a transcription factor co-activator that is a key regulator of both hepatic gluconeogenesis and SeP biosynthesis<sup>(172)</sup>.

The diabetogenic effect of Se may explained by another mechanisms that could need further support. Number of studies have focused on the insulin antagonistic protein tyrosine phosphatase 1B (PTP1B) as a molecular target for the treatment of obesity and insulin resistant diabetes<sup>(173)</sup>. In studies with humans<sup>(174)</sup> and in animal models, it could be shown that PTP1B deficiency, obtained by a lowered expression<sup>(175-177)</sup> or biochemical enzyme inhibition<sup>(157)</sup>, protects from obesity and insulin resistance, whereas high PTP1B activities can accelerate these diseases<sup>(178)</sup>. The long term supplementation of rats with Se cause up-regulation of the insulin antagonistic PTP1B<sup>(179)</sup>. An increased PTP1B activity may promote diabetes by reducing insulin signaling at the receptor level which may interpret the lower level of activated phosphor-IR in the present study. All of these effects impair the insulin signaling at different levels which end results with decline in the level of Glut4 that responsible for glucose uptake. These results are in agree with the result of Yuan and Liu<sup>(180)</sup> who showed that the reduction in the Glut4 levels contributes significantly to the elevated glucose level.

The disturbed glucose and insulin homeostasis as a result of long-term supplementation with Se compounds is associated with mild change in the lipid profile. While very mild change in the total cholesterol level was observed with the two Se compounds, only sodium selenate significantly decrease triglycerides level at low dose

(10µg/kg) and significantly increase its level at the highest dose (100 µg/kg). In sodium selenate treated rats, the triglyceride level is positively correlated with seP in the liver and with Se-GPx in the liver and muscle, also, the two Se compounds have no significant effects on the LDL-cholesterol but significantly increase the level of HDL-cholesterol which imply a mild or even a good effect of long-term Se supplementation on the lipid profile.

The idea to regard high Se intake as a risk factor for type 2 diabetes may appear paradoxical, bearing in mind existing evidence for insulin-mimetic and anti-diabetic actions of inorganic Se compounds such as sodium selenate and sodium selenite <sup>(181,182)</sup>. Moreover, the antioxidant ROS-detoxifying capacity of selenoenzymes suggests that Se may protect against late complications of diabetes caused by chronic oxidative stress <sup>(95)</sup>. Although the use of antioxidants appears to be a logical approach in the therapy of diabetes, its success has not been proven so far <sup>(183)</sup>, which might be related to this dual role of intracellular ROS in modulation of insulin signaling. In healthy humans, high doses of dietary antioxidant supplements can impair the sensitivity of the skeletal muscle for insulin, as it has been observed after daily combined administration of 1 g vitamin C and 400 IU vitamin E for 4weeks <sup>(99)</sup>. Interestingly, older adults with a relatively low selenium status did not show any diabetogenic effects after a six-month supplementation <sup>(184)</sup>. These results suggest that selenium in excess, not selenium itself, may be the reason for the increased risk found in those with a high selenium status and intake. This underscores the U-shaped risk response curve of selenium intake, which is further verified by the animal study that showed that both selenoprotein deficiency and a high expression of selenoprotein cause diabetogenic effects <sup>(185)</sup>. It's possible that taking extra selenium overcomes the natural balance. Perhaps excess selenium has a negative effect on the endocrine system."

Rats treated with 5 µg/kg in both sodium selenate and selenocysteine groups show no significant differences from control in all parameters .

The results of the present study provide evidence that the Se compounds; sodium selenate and selenocysteine can impair the insulin secretion and insulin sensitivity in liver, skeletal muscle and adipose tissues. On the other hand, for consumers of Se supplements it may be of interest to know that selenocysteine as a widely used Se compound in dietary supplements did not interfere with insulin secretion as sodium selenate.