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Research Article

Selenium Supplementation Increases Hepatic Glucose-6-Phosphatase and Peroxisome Proliferator Activated Receptor Gamma Coactivator-1α Activity in Male Wistar Rats

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Summary: Increased selenium supplementation has been implicated in diabetes mellitus via peroxisome-proliferator-activated-receptor-gamma-coactivator-1-alpha (PGC-1α) associated pathways. This study was designed to investigate the effect of selenium supplementation on PGC-1α and glucose-6-phosphatase (G6Pase) as well its likely hepato toxicity in male Wistar rats. Animals were randomly divided into 3 groups (n=10/group) and treated orally with water (0.2ml - group 1) or selenium (25µg/day -group 2; 50µg/day - group 3) for 28 and 56days, respectively. Thereafter, blood samples were collected and estimated for glucose, alkaline-phosphate (ALP), gamma-glutamyltransferase (GGT) and aspartate-aminotransferase (AST). Liver homogenates were analyzed for PGC-1α and G6Pase activity. Significant dose-dependent increases in blood glucose, hepatic PGC-1α and G6Pase activities were observed on days 28 and 56 in selenium groups compared to group 1. Serum GGT activity increased in both selenium groups on day 28 however, on day 56 values in group 2 were reduced and increased in group 3, respectively. Compared to control ALP reduced in selenium groups while AST was not significantly different. This study suggests that selenium supplementation increases hepatic peroxisome-proliferator-activated-receptor-gamma-coactivator-1α and glucose-6-phosphatase activity leading to a likely increase in hepatic glucose output. It also shows that though selenium supplementation at the doses used maybe nontoxic to hepatocytes, it may however exert potential toxicity on the biliary tract.

Keywords*: Selenium, Hepatic peroxisome proliferator activated receptor gamma coactivator-1-alpha, Hepatic glucose-6-phosphatase, Liver enzymes*

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INTRODUCTION

Micronutrients are essential inorganic substances needed by the body in small quantities for important processes such as growth, bone and teeth formation, brain development, immune functions and glucose homeostasis (Soetan *et al*, 2010). They are also essential components of enzyme systems and are often required for normal nerve function and development. Examples of these micronutrients include calcium, phosphorus, magnesium, sodium, potassium, chloride, iron, zinc, iodine, selenium and copper (Gernand *et al*, 2016).

Selenium is a micronutrient that is critical for the synthesis of selenoproteins, which play important roles in the antioxidant defense system, reproduction, normal muscle function, tumor prevention, and tumor suppression (Mehdi *et al*, 2012). According to Institute of Medicine, Food and Nutrition Board, Washington DC (2000) and Ogawa-Wong *et al*., (2016) the recommended daily average for selenium is 55 microgram and its supplementary intake has long been publicized, due to its reported cytoprotective properties which arises from its ability to up-regulate antioxidant selenoenzymes (Mehdi *et al*, 2012) and inhibit many inflammatory cell mechanisms (Huang *et al*, 2012). Selenium has also been reported to exert hormesis effects with low doses having beneficial effects and a high dose having toxic effects (Huang *et al*, 2012). Selenium supplementation may have adverse effects in people who are already receiving an adequate intake of selenium (Ogawa-Wong *et al*, 2016). Individuals with high baseline of Selenium have been reported to have an increased risk of type-2-diabetes development (Stranges *et al*, 2007; Vinceti *et al.*, 2018). The actual potential mechanisms underlying this association between selenium status and type 2 diabetes are profuse (McClung *et al*., 2004; Misu *et al*., 2010; Steinbrenner, 2013; Zhou *et al*., 2013; Ishikura *et al*., 2014) however, increased activity of peroxisome proliferator activated receptor gamma coactivator 1 alpha (PGC-1α) has been proposed to be a strong link between high selenium

intake and type-2-diabetes mellitus (Steinbrenner *et.al*, 2011).

PGC-1α is a transcription co-activator which interacts with a broad range of transcription factors to regulate a wide range of biological responses including adaptive thermogenesis, mitochondrial biogenesis, glucose and fatty acid metabolism (Liang and Ward, 2006). It has been reported to control the transcription of rate-limiting gluconeogenic enzymes such as glucose-6-phosphatase. Glucose-6-phosphatase is found mainly in the liver and plays an important role in regulating hepatic glucose output (Wu *et al*, 2016). This study was therefore designed to investigate the effect of sub-chronic and chronic selenium supplementation on hepatic peroxisome proliferator activated receptor gamma co-activator 1 alpha (PGC-1α) and glucose-6-phosphatase activity as these have been observed to regulate hepatic glucose output. The effects of selenium supplementation on the liver toxicity will also be evaluated using the liver function tests

MATERIALS AND METHODS

Animal and grouping: Thirty male (30) Wistar rats were housed in standard well-aerated laboratory cages. They were fed on standard rat chow (Ladokun feeds, Nigeria) and allowed free access to drinking water *ad libitum.* The Applied and Environmental Physiology Unit, Department of Physiology, University of Ibadan approved this experiment. Animals received humane care, and procedures were in accordance with the Guide for the Care and Use of Laboratory Animals (1996, published by National Academy Press, 2101 Constitution Ave. NW, Washington, DC 20055, USA). The animals were randomly divided into 3 groups of 10 rats each consisting of Group 1, that was given distilled water (0.2ml); group 2 which were treated with selenium (as Sodium selenite) at 25µg/day and group 3 that were treated with selenium at -50µg/day (Kang *et al*., 2001), respectively. All treatments were carried out orally once daily for 28 and 56days respectively.

Measurements and Biochemical Assay

Body weight and Assessment of Blood Glucose level: Body weight was monitored throughout the study using a standard laboratory scale while blood glucose, before and after Selenium supplementation, was assessed using the tail tipping method as follows: the tail tip of the animal was punctured with a disposable blood lancet. A drop from the pool of blood on the tip of the tail was collected on an Accu-Check active glucose test strip and thereafter analyzed on an Accu-Check active glucometer (Tack *et al*., 2012) (Roche, Germany), which uses the glucose oxidase method (Barham and Trinder, 1972) as its method of analysis.

Biochemical Assays

Blood samples were collected by cardiac puncture under mild anesthesia (Sodium thiopentone-50mg/kg) into plain sample bottles. The samples were allowed to coagulate and centrifuged at 3500rpm for 15minutes to obtain serum. Aliquots of the clear serum obtained was analyzed for alkaline phosphate (ALP), Aspartate Aminotransferase (AST), and gamma-glutamyltransferase (GGT) respectively.

Assessment of Alanine Aminotransferase (ALT) activity: The ALT activity was determined following the principle described by Reitman and Frankel (1957). Briefly, 0.1ml of diluted serum was mixed with phosphate buffer (100mmol/L, pH 7.4), L-alanine (100mmol/L), and α- oxoglutarate (2mmol/L) and the mixture was incubated for exactly 30mins at 37^0C. 0.5ml of 2,4- dinitrophenylhydrazine (2mmol/L) was then added to the reaction mixture and allowed to stand for exactly 20mins at 25^0C. Thereafter, 0.5ml of NaOH (0.4mol/L) was added and the absorbance was read against reagent blank after 5mins. Reagent blank was prepared as described above replacing sample with 0.1ml of distilled water. The ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine at 546nm.

Assessment of Aspartate Aminotransferase (AST) Activity: The AST activity was determined following the principle described by Reitman and Frankel (1957). Briefly, 0.1ml of diluted sample was mixed with phosphate buffer (100mmol/L, pH 7.4), L-aspartame (100mmol/L), and α-oxoglutarate (2mmol/L) and the mixture incubated for exactly 30mins at 37^0C. 0.5ml of 2,4-dinitrophenylhydrazine (2mmol/L) was added to the reaction mixture and allowed to stand for exactly 20min at 25^0C. Then 5.0ml of NaOH (0.4mol/L) was added and the absorbance read against the reagent blank after 5mins at 546nm. The AST activity was measured by monitoring the concentration of oxaloacetate-hydrazone formed with 2, 4- dinitrophenylhydrazine.

Assessment of gamma-glutamyltransferase (GGT) Activity: The GGT activity was determined following the principle described by (Szasz, 1974) in which the rate of increase in absorbance due to release of p-nitroaniline is measured at 405 nm and 37°C. Briefly, 2.90 ml of the substrate solution (0.16M Glycylglycine, 0.016M Magnesium chloride, 0.05M Tris base, 120mg Gamma-glutamyl-p-nitoanilide) was pipetted into a cuvette and incubated in the spectrophotometer at 37°C for 10 minutes to attain temperature equilibration. A blank recording was

taken at 405nm and curve of the change in absorbance per min ($E_{405/min}$) was plotted. 10ml of the sample was thereafter added to the cuvette, mixed and the increase in absorbance at 405nm was taken for 5-8min. The change in absorbance ($\Delta E_{405\ nm/min}$) was calculated from the linear portion of the curve.

Determination of hepatic glucose-6-phosphatase and PGC1-α activity: Liver samples were also excised from each animal and homogenized on ice with ice-cold 0.25M sucrose buffer (for determination of glucose-6-phosphatase activity) and 0.1 M phosphate buffer (1: 4 w/v, pH 7.4) (for determination of PGC1-α activity), respectively. The homogenates obtained was centrifuged at 4000 RPM for 10 minutes at 4^0C and the resulting supernatants was frozen at -4°C until use. Aliquot of the supernatants was assayed for glucose-6-phosphatase activity using the method of Koide and Oda (1959). Briefly, into a test tube, 300µl of 0.1M citrate buffer, 500µl of 150mM glucose-6-phosphate solution and 200µl of sucrose buffer extracted liver homogenate were added. The mixture was thoroughly mixed and incubated at 37^0C for 1 hour. Thereafter 1.0ml of 10% trichloroacetic acid (TCA) was added to stop the reaction and placed on ice. After 10mins on ice, the mixture was centrifuged. 1ml aliquot of the supernatant was pipetted into a test tube to which was added 1.0ml of 1.25% Ammonium molybdate and 9% Ascorbic acid in a stepwise manner. The mixture was incubated at room temperature for 1hour and read at 660nm against a reagent blank. The liberated phosphate was determined by comparing with standards of known concentration.

Aliquots of the supernatant were also evaluated for Hepatic PGC1-α activity using Enzyme-Linked Immunosorbent Assay (ELISA) kits according to the manufacturers (Bioassay Technology Laboratory, China.) directions.

Statistical Analysis

All the data were presented as Mean + Standard Error of Mean (SEM) and subjected to one-way analysis of variance (ANOVA) and Newman-Keil post analysis test using the GraphPad prism version 7.0 (GraphPad software, San Diego, CA USA). Statistical significance was taken at P<0.05.

RESULTS

Effects of selenium supplementation on the body weight (g) and blood glucose level (mg/dL) in control and experimental groups: Body weight in the control group increased consistently with values obtained on day 56 being 7.3% higher compared to day 0 values within same group. Animals in group 2 (Selenium 25µg treated) and 3 (Selenium 50µg treated) also showed body weights that increased consistently with values on day 56 being 19.6% and 20.1% increased (p<0.05) compared with initial values (day 0) within each group respectively (Table 1). Blood glucose values obtained in the control group was relatively stable throughout the study while values in group 2 and 3 increased consistently with values obtained on day 56 being 34.2% and 67.8% increased (p<0.05) respectively compared to their initial day 0 values (Table 2).

Table 1.
Effect of selenium on the body weight (g) in control and experimental groups

	Control	Group 2 (selenium 25µg treated)	Group 3 (selenium 50µg treated)
Day 0	172.0 ± 5.3	171.6 ± 6.5	179.2 ± 7.3
Day 28	180.8 ± 5.2	197.6 ± 2.2	207.0 ± 3.4[a]*
Day 56	184.6 ± 5.7	205.2 ± 1.5[b]*	215.2 ± 1.6[b]*

** Indicates values that are significantly different from day 0 values within same group.[a] indicates values that are significantly different from control on day 28; [b] indicates values that are significantly different from control on day 56.*

Table 2.
Effect of selenium on blood glucose level (mg/dL) in control and experimental groups

	Control	Group 2 (selenium 25µg treated)	Group 3 (selenium 50µg treated)
Day 0	42.0 ±8.1	47.4. ±2.9	49.0 ±2.7
Day 28	50.8 ±2.7	66.6 ±4.5[a]*	71.0 ±4.8[a]*
Day 56	54.6 ±4.5	63.6 ±2.8[b]*	82.2 ±5.7[b]*

** Indicates values that are significantly different from day 0 values within same group. [a] indicates values that are significantly different from control on day 28; [b] indicates values that are significantly different from control on day 56.*

Effects of selenium supplementation on hepatic glucose-6-phosphatase and peroxisome proliferator activated receptor gamma coactivator-1α in control and experimental groups: Glucose-6-phosphatase (G6Pase) activity (µmol Pi liberated/hour/mg protein) was significantly increased (p<0.05) on day 28 and 56 in group 2 (Selenium 25µg treated) and 3 (Selenium 50µg treated) compared to group 1 (control). However, G6Pase values on day 56 in groups 2 (82.8±3.8) and 3 (260.9±12.9) were significantly reduced compared to values obtained in same groups on day 28 (196.4±6.8; 311.5±15.0), respectively (Fig. 1).

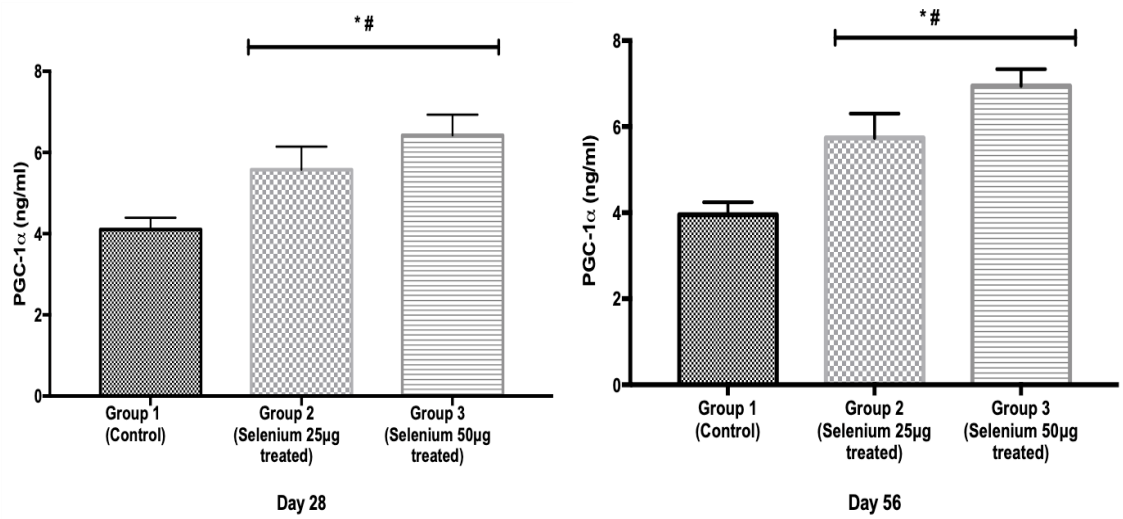

Figure 1.
Effect of Selenium on hepatic glucose-6-phosphatase activity in control and experimental groups.
** Indicates values in group 2 that are significantly different from group 1 . # Indicates values in group 3 that are significantly different from group 1; ª indicates values in groups 2 and 3 on day 56 that are significantly different from values obtained in same group on day 28.*

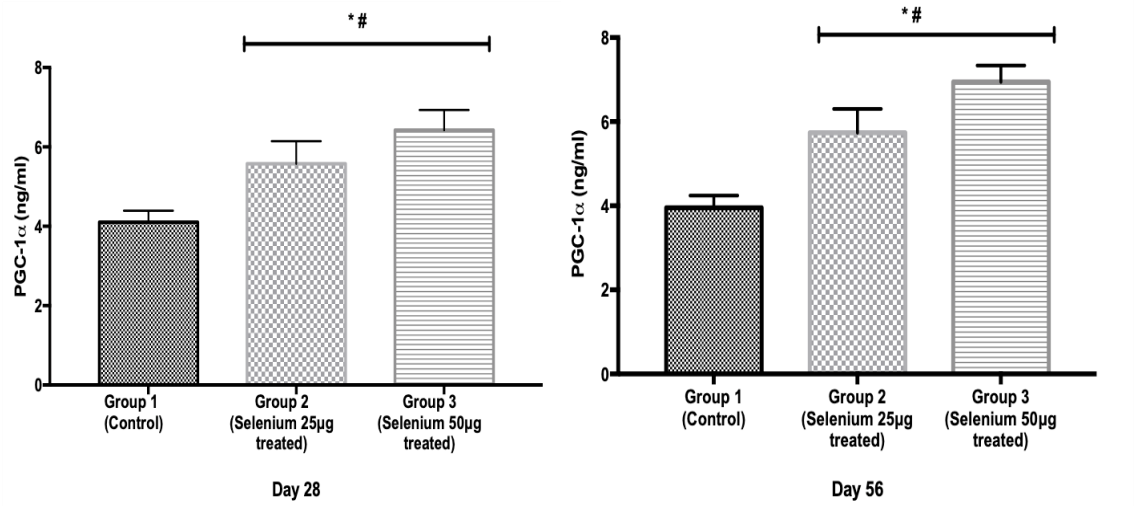

Figure 2.
Effect of Selenium on peroxisome proliferator activated receptor gamma coactivator-1α(PGC1 alpha) activity in control and experimental groups.
** Indicates values in group 2 that are significantly different from group 1. # Indicates values in group 3 that are significantly different from group 1.*

Table 3.
Effect of selenium on Liver function test in control and experimental groups

	Alkaline Phosphate (U/L)		Gamma-glutamyltransferase (U/L)		Aspartate Aminotrasferase (U/L)	
	Day 28	**Day 56**	**Day 28**	**Day 56**	**Day 28**	**Day 56**
Group 1 (Control)	120.0 ±1.8	125.4 ±2.14	1.74 ±0.26	1.55 ±0.35	44.6 ±0.68	47.4. ±2.9
Group 2 (Selenium 25µg treated)	99.0 ±5.50*	111.8 ±2.60*	2.66 ±0.21*	0.96±0.26*	41.0 ±1.76	42.6 ±2.54
Group 3 (Selenium 50µg treated)	112.2 ±2.13#	112.6 ±1.44#	3.00 ±0.23#	2.80 ±0.25#	40.6 ±1.36	43.6 ±1.12

** Indicates values in group 2 that are significantly different from group 1 (control). # Indicates values in group 3 that are significantly different from control.*

Peroxisome proliferator activated receptor gamma coactivator-1α activity (ng/ml) was increased significantly ($p<0.05$) on day 28 and 56 in groups 2 (5.58±0.57; 5.74±0.57) and 3 (6.42±0.51; 6.94±0.39) compared to group 1 (4.10±0.29; 3.95±0.35) on both days respectively (Fig. 2).

Effects of selenium supplementation on liver function tests in control and experimental groups: Alkaline phosphate (U/L) on days 28 and 56 significantly reduced in groups 2 (Selenium 25µg treated) and 3 (Selenium 50µg treated) compared with group 1(control) respectively (Table 3). On days 28 and 56, gamma-glutamyl transferase (U/L) values indicate a significant increase in the experimental groups compared to control (Table 3). However, aspartate aminotransferase (U/L) values on day 28 and 56 in all groups were not significantly different (Table 3).

DISCUSSION

Selenium supplementation in humans has been widely advocated because it is a vital constituent of selenoproteins, which play critical roles in reproduction, thyroid hormone metabolism, DNA synthesis, prevention of oxidative damage and protection against infections (Mehdi *et al*, 2013). However, the likely predisposition of excessive Selenium supplementation to diabetic conditions has been suggested (Steinbrenner *et al*, 2011). This study showed an increase in the body weight of selenium treated animals after sub-chronic (28days) and chronic (56days) supplementation at both low (25µg/day) and high (50µg/day) doses. This is consistent with the previous finding of Hawkes and Keim (2003) who affirmed that high Selenium diet induces subclinical hypothyroidism, which often leads to decreased energy expenditure and increased weight gain. The percentage increase in blood glucose level in the Selenium treated groups compared to control is consistent with the report of Ayaz *et al*, (2005) and suggests a likely hyperglycemic effect of Selenium supplementation which could be dose dependent as treatment with the selenium (50µg/day) treatment group having a higher glucose level than that of the lower dose (selenium, 25µg/day) treatment group.

This study also shows an increase in PGC-1α activity after sub-chronic and chronic selenium supplementation. This is consistent with the reports of Mehta *et al*, (2012) who also reported a significant increase in PGC-1α activity with sodium selenite treatment. An increase in hepatic PGC-1α activity and expression has also been linked to the stimulation of increased hepatic glucose output, and when coupled with its reported inhibitory effect on insulin signaling and secretion (Wu *et al*, 2002; Koo *et al*, 2004; Liang and Ward, 2006) may also account for the increase in blood glucose level observed in the selenium treated groups compared to controls. Furthermore, glucose 6-phosphatase - an enzyme that is found mainly in the liver and kidney – is known to hydrolyze glucose 6-phospate resulting in the creation of a phosphate group and free glucose (Ghosh *et al*., 2002). The activity of this enzyme was increased in the selenium treated groups in this study thus suggesting increased hepatic glucose production and hence the observed differences in blood glucose level between control and selenium treated groups, respectively. Taken together, the increased liver PGC-1α activity, glucose 6-phospatase activity and blood glucose observed in this study suggests a potential diabetogenic activity of selenium supplementation as has been reported in a large number of epidemiologic studies (Vinceti *et al.*, 2018).

This study investigated the likelihood of the existence of a liver disease following acute and chronic selenium supplementation at low (25µg/day) and high doses (50µg/day), respectively. The results obtained in the present study indicates a reduced alkaline phosphate (ALP) level in the selenium treated groups compared to control, which suggests that, the hepatocytes and osteoblasts were not damaged by selenium supplementation (Thapa and Anuj, 2007). However, the increased gamma-glutamyl transferase (GGT) levels observed in the selenium treated animals suggests the likelihood or presence of bile duct problems as it is usually the first liver enzyme to rise in the blood when any of the bile ducts that carry bile from the liver to the intestines become obstructed (Lum and Gambino, 1972;Singh *et al*, 2006). Aspartate aminotransferase (AST) levels were also not significantly different across the groups and this again suggests that selenium supplementation may not be toxic to either liver or muscle cell at the doses used.

In conclusion, this study suggests that sub-chronic and chronic selenium supplementation may increase the activity of both peroxisome proliferator-activated receptor gamma coactivator-1alpha (PGC-1α) and glucose 6-phosphatase leading to an increase in hepatic glucose output. The increased production of gamma-glutamyltransferase (GGT) observed in this study suggests a likely predisposition to biliary track dysfunction following sub-chronic and chronic selenium supplemental intake.

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