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Selenite supplementation modulates hepatic metabolic sensors AMPK and SIRT1 in binge drinking exposed adolescent rats by avoiding oxidative stress.

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Short Title: Selenite supplementation modulates hepatic metabolism in binge drinking.

ABSTRACT:

Binge drinking (BD) is the main alcohol consumption pattern among teenagers. Recently, oxidative stress (OS) generated by BD exposition has been related to hepatic metabolic deregulation and cardiovascular dysfunction. This study analyzes if BD by generating oxidation modulates the alteration in hepatic energy homeostasis through two important regulators of energy metabolism: the NAD+ -dependent sirtuin deacetylase (SIRT1) and AMP-activated protein kinase (AMPK); and if supplementation with the antioxidant selenium (Se) improves those metabolic disorders. Four groups of adolescents rats, supplemented or not, with Se (0.4 ppm) and exposed an intermittent i.p. BD model had been used. BD rats had increased AST/ALT ratio and total bilirubin in serum as well the lipid peroxidation in the liver. Also, these BD rats had higher abdominal-thoracic ratio and increased values of TG, gluc, and chol than control group, provoking an increase in mean blood pressure (MBP). This alcohol consumption pattern decreased hepatic Se deposits, cytoplasmic GPx activity, and GSH levels as well as expressions of two metabolic sensors and the pAMPK/AMPK ratio. Se supplementation restored antioxidant parameters and decreased lipid oxidation, avoiding OS and improving the hepatic expression of pAMPK and SIRT1, contributing to improving metabolic (better lipid profile and IRS-1 expression) and vascular function (lower MBP), and to increase hepatic functionality (lower AST/ALT ratio). All these actions decrease cardiometabolic risk factors development in the short and long term and could disrupt the relationship between BD and MS, two problems which are currently affecting adolescents.

Keywords: Binge drinking, energy homeostasis, metabolic sensors, oxidative stress.

1. INTRODUCTION:

Alcohol intermittent binge drinking (BD) is the alcohol consumption's pattern of greatest concern among teenagers[1]. Adolescent BD exposition is associated not only with a range of acute alcohol-related nervous harms[2], but also to long term systemic harms related to hepatic[3], renal[4] and cardiovascular[5] damage. Acute ethanol, contrary to chronic moderate consumption, greatly induces cytochrome P450 2E1 (CYP2E1) activity, generating a great amount of oxidative stress (OS). CYP2E1 is a powerful generator of reactive oxygen species (ROS) needed to metabolize ethanol in high doses[6,7]. Moreover, it has been shown that BD exposition during adolescence alters the activity of the main endogenous antioxidant enzymes[3]. The OS generated by BD exposition, has recently been related to a metabolic deregulation process which affects the energy homeostasis mainly in liver, even during early growing states[8]. In adults, it leads to fatty liver disease, steatosis, high triglycerides (TG), cholesterol (chol) and glucose (gluc) serum levels, insulin resistance (IR) and hypertension (HTA)[9–11]. Most of these alterations are factors of metabolic syndrome (MS), a highly prevalent disease in adolescents [12].

Alcohol produces these alterations in the energy balance by affecting, among others, two important regulators of energy metabolism: the NAD+ -dependent sirtuin deacetylase (SIRT1) and AMP activated protein kinase (AMPK)[13]. SIRT1 produces genetic changes that mediate the increase in longevity caused by calorie restriction; its overexpression reduces the incidence of cardiovascular and metabolic disorders. When a cell's energy state is diminished, AMPK activation restores energy balance by stimulating catabolic processes that generate ATP and downregulating anabolic processes that consume ATP[14]. Recently, it has been described that they both have similar effects on several processes such as cell energy metabolism, inflammation, or mitochondrial function, and that their dysregulation predisposes to disorders such as IR[15].

Alcohol, even in the form of BD, is known to affect SIRT1 as it decreases the NAD+/NADH ratio, on which SIRT1 is dependent[16]. On the other hand, different works establish that alcohol

decreases the phosphorylation and activation of AMPK[17–20]. It seems that during the oxidation of NADH, produced by the metabolism of alcohol, energy generated is used to synthesize ATP; therefore, the AMP/ATP ratio is decreased and AMPK inhibited[21]. Alcohol also affects these molecules indirectly by the OS generated as a consequence of the repeated acute alcohol exposition. ROS are not only critical factors that control the activation of AMPK[22] and inhibit SIRT1[23], but also they are known to play a fundamental role in IR or MS[24,25]. In fact, Jiang et al.[18] using an acute ethanol model of alcoholization in adult rats, have demonstrated that the impaired Adiponectin-SIRT1-AMPK signaling pathway contributes, at least in part, to the development of alcoholic fatty liver disease, affecting different enzymes related to lipogenesis. According to that, different natural antioxidants have been used in ethanol exposed animals in order to activate AMPK and prevent hepatic steatosis [24,26,27]. Probably, these antioxidants also have actions on SIRT1, since it is known, for instance, that the antioxidant resveratrol used SIRT1 to activate AMPK [28].

Ojeda et al.[3], have found in BD adolescent rats a disruption in the homeostasis of the antioxidant Selenium (Se). Se plays a key biological antioxidant role being the catalytic center of different selenoproteins such as Glutathione Peroxidase (GPx). The GPx family members play different roles; in general, they act as antioxidants, reducing free hydrogen peroxide to water in different cells and organelles, with an important role in mitochondrial survival and endoplasmic reticulum function[29]. GPxs also have been implicated in the modulation of the transcriptional factor NF-κB protein, having a role in immune and apoptotic responses[30,31]. Se and GPxs have also been directly related to IR process, AMPK activation, hepatic steatosis and vascular injury, being this effect intimately related to Se dose and oxidative balance[32–34]. Recently, Yi et al.[35] have described that Se-enriched diet avoids in part the hepatic damage caused by chronic alcohol and high fat diet consumption. The same authors concluded that Se inhibited lipid accumulation in hepatocytes; improved dyslipidemia; decreased OS; and regulated lipid metabolism related genes such as AMPK, PPAR-α and SREBP1, avoiding lipogenesis and inflammation.

The aim of this study is to find whether BD exposition during adolescence leads to changes in the hepatic energetic sensors AMPK and SIRT1 leading to metabolic disorders, and if these sensors' alterations depend on OS generated by BD exposition. Secondly, it will be analyzed if Se supplementation, as a potent hepatic antioxidant, can modulate these proteins' expression, improving the BD exposed rats' metabolic profile, and consequently, avoiding cardiometabolic risk factors. The association between BD and individual components of MS among the

adolescent population has not been studied in great detail. Therefore, results which support a relationship among them, for instance by generating oxidation or affecting key regulators of energy metabolism, will stimulate research for antioxidant substances, such as Se, that could improve cardiovascular health during adolescence.

2. MATHERIAL AND METHOD:

2.1. Animals.

For these experiments we have used forty adolescent male Wistar rats from the Centre of Production and Animal experimentation, University of Seville (CITIUS-3). Rats were received at postnatal day (PND) 21 and allocated in groups of two rats per cage for one week in housing conditions. Corresponding to the adolescent period in Wistar rats, the experimental treatment began when the rats reached PND 28 and ended at PND 47, lasting 3–weeks. Then, rats were randomly divided into four groups (n=10/group) according to their treatments. Groups were: control group (C): which received standard pellet diet and drinking water ad libitum, and on the corresponding days, an isotonic physiological saline solution (PSS) intraperitoneally (i.p.); BD alcohol group (BD): which received standard pellet diet and drinking water ad libitum, and on the corresponding days, an ethanol solution in PSS i.p.; control Se group (CS): which received standard pellet diet and Se supplementation in drinking water ad libitum, and on the corresponding days an injection of PSS; and BD alcohol Se group (BDS): which received standard pellet diet and drinking water supplemented with Se ad libitum, and on the corresponding days, an alcohol solution in PSS i.p.

Standard pellet diet (2014 Teklad Global 14% Protein Rodent Maintenance Diet, Harlan Laboratories, Barcelona, Spain) contains 0.23 ppm of Se. The Se supplemented groups (CS and BDS) received 0.14 ppm of extra Se as anhydrous sodium selenite (Panreac, Barcelona, Spain) in drinking water during all the experimental period. Drinking water was purified by reverse osmosis system, and Se was detected by our PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectrometer. The amount of 0.14 ppm of Se was chosen taking into account the amount of Se consumed by adolescent rats just with the Standard pellet diet, and based on the study of Yang et al [36], which, using sodium selenate as the Se source, reported that GSH-Px activities in rats' plasma and liver were maximized at 500 μg/kg dietary Se. Since the C adolescent rats used in this study have an intake of approximately 12 g of food per day, with a supplemented Se diet of 0.5 ppm they will receive 6 μg/day. With the objective of studying the effects of excessive but not extreme Se doses, Se supplemented water was calculated in order not to reach 6 μg/day of Se intake. The Se supplemented protocol used in

this study leads to a significant repletion of liver Se deposits ($p<0.001$). This fact has been proved in a previous paper from our lab using similar experimental rats (C: 0.21 ± 0.012; BD: 0.15 ± 0.009; CS: 0.29 ± 0.012; BDS: 0.27 ± 0.008 µg/g dry weight) [3].

As always in our lab, during the whole experimental protocol rats were kept at an automatically controlled temperature (22-23 ºC) and in a 12-hour light-dark cycle (09:00 to 21:00). Animal protocols were approved by the Ethics Committee of the University of Seville, and performed in accordance with EU regulations (Council Directive 86/609/EEC, November 24th 1986).

2.2. Nutritional control

During the whole experimental period body weight and solid and liquid intakes were monitored daily at 9:00 a.m. to avoid changes due to circadian rhythms. The amount of food and water ingested were calculated by the difference between their values every morning and the following one. Total kilocalories consumed were estimated by multiplying the grams of food consumed by 4.1 calories. Daily Se intake was calculated by multiplying Se concentration in standard pellet (0.23ppm) by the grams of food consumed, and multiplying Se concentration in supplemented water (0.14 ppm) by the ml of water ingested.

At the end of the experiment, prior to the sacrifice, the cranium-caudal length was measured using a metric caliper. Body mass index (BMI) was estimated by the formula: Body weight (g)/length^2 (cm^2). At that moment, the abdominal circumference value (immediately anterior to the hind foot), the thoracic circumference one (immediately behind the foreleg) and their ratio were also determined.

2.3. Ethanol treatment

BD and BDS groups received an i.p. injection of alcohol (20 % v/v) in PSS (3 g/Kg/d) at 7:00 p.m., when the dark cycle began. These injections were administered for 3 consecutive days each week, during 3 weeks. This experimental protocol has been used previously by this lab Ojeda et al . [37], proving that one hour after the i.p. administration the highest peak of blood alcohol concentration was determined, which reached 125.0±9.8 mg/dl. In parallel with alcohol injections, C and CS groups received an i.p. injection of an equal volume of PSS.

2.4. Samples

After 12h of the last injection, rats were transferred to individual metabolic cages and fasted for 12h; urine sample was collected. Therefore, 24h after the last treatment, animals

were anesthetized with an i.p. injection of 28% w/v urethane (0.5 ml/100 g of body weight). Blood samples were obtained by heart puncture and collected in tubes**.** Serum samples were prepared using low-speed centrifugation for 15 minutes at 1300 x g. In order to obtain hepatic samples, the abdomen was opened through a midline incision. Liver was then removed, debrided of adipose and connective tissue in ice-cold saline, weighed, frozen in liquid nitrogen and stored at −80 ºC. Hepatic somatic index (HIS) was estimated as hepatic weight/body weight × 100.

2.5. Biochemistry parameter analysis

In serum, transaminases (alanine aminotransferase (ALT) and aspartate aminotransferase (AST)), total bilirubin, glucose (gluc), cholesterol (chol), and triglycerides (TG) were measured with an automated analyzer (Technicon RA-1000, Bayer Diagnostics). The albumin levels in urine were spectrophotometrically determined using commercially available kits.

2.6. Selenium Analysis.

According to previous paper, serum samples were diluted five-fold in 0.2% v/v HNO_3 and 0.2% Triton X-100 solutions Ojeda et al.[38] Then serum Se levels were analyzed by using a PerkinElmer AAnalyst™ 800 high-performance atomic absorption spectrometer with a Transversely Heated Graphite Furnace (THGA) (PerkinElmer, Ueberlingen, Germany). The sources of radiation were electrodeless Se discharge lamps (EDLs). The instrumental operating conditions and the reagents are the same that we have used in the previous paper Ojeda et al.[38].

2.7. Lipid oxidation.

Serum protein content was estimated by the method of Lowry et al.[39] The peroxidation of lipid in serum samples was detected by the method based on the reaction between malondialdehyde (MDA) and thiobarbituric acid (TBA)[40]. The absorbance of the pink supernatant was measured at 535 nm and results were expressed as moles of MDA per milligram of protein (mol/mg protein). Serum MDA results were used to estimate the ratio serum Se/MDA.

2.8. Blood Pressure.

Systolic and diastolic blood pressure (SBP and DBP) were monitored with pressure meter (NIPREM 645, CIBERTEC, Spain) using the indirect tail occlusion method. Measurements were taken 24 hours after the last experimental injection in conscious animals. The signals collected

were treated with an IT support via a data acquisition system coupled to the pressure meter. Mean blood pressure (MBP) was calculated from SBP and DBP data: MBP = [SBP + (2 X DBP)] / 3.

2.9. Proteins immunoblotting assays.

The expression of the hepatic proteins IRS-1, total AMPK (tAMPK), phosphorylated AMPK (pAMPK) and SIRT1 in adolescent rats was detected by Western Blotting. Before it, liver samples were homogenized (100xg for 1min, 1:4 w/v) using a Potter homogenizer (Pobel 245432, Madrid, Spain) in phosphate buffer (1/10 p/v with K_2HPO_4 (PANREAC) 50mM; KH_2PO_4 (PANREAC) 50mM and EDTA (SIGMA) 0,01mM) on an ice bath. Previously a protease inhibitor (Complete Protease inhibitor Cocktail Tablets, ROCHE) was added. The homogenate was centrifuged at 1400xg for 10 minutes at 4 ºC, and the supernatant was used for the determinations. The protein concentration of all samples was analyzed by the method of Lorry et al. [39].

Liver samples for Western Blotting determinations contained 100 µg of protein. Proteins from the liver were separated on a polyacrylamide gel and were transferred onto a nitrocellulose membrane (Immobilon-P Transfer Membrane, Millipore, Billerica, MA, USA) using a blot system (Transblot, BioRad CA, USA). They were incubated overnight at 4ºC with specific primary antibodies (rabbit polyclonal IgG, Santa Cruz Biotechnology) against IRS-1 (1:1000), tAMPK (1:4000), pAMPK (1:2000) and SIRT-1 (1:2000). Secondary antibody (anti-rabbit IgG HRP conjugate, Santa Cruz Biotechnology) was incubated in dilutions of 1:5000 for IRS-1, 1:10000 for tAMPK, 1:2000 for pAMPK and 1:5000 for SIRT1. Monoclonal mouse anti β-actin (IgG1 A5441, Sigma-Aldrich, Spain) at 1:4000 was used to detect β-actin as a loading control, followed by the secondary antibody anti-mouse IgG Peroxidase conjugate (A9044, Sigma-Aldrich, Spain) at 1:8000. The signals were detected using enhanced chemiluminescence Luminol ECL reagent (GE Health Care and Lumigen INC Buckinghamshire, UK). Relative density of the bands was determined by Image J software (Java-based image-processing and analysis software). The results were expressed as percent arbitrary relative units, referring to the values in control animals.

2.10. Statistical Analysis

Data are expressed as Mean ± SEM (standard error of the mean) and analyzed by using GraphPad InStat 3 (CA, USA) statistical analysis software. Difference was assessed using analysis of variance (one-way ANOVA). The number of samples used to obtain each final data

was n=8. A p value <0.05 was considered statistically significant. When ANOVA resulted in differences, multiple comparisons between means were studied by the Tukey-Kramer test.

3. RESULTS:

There were no differences in Kcal intake or body weight increase among the 4 experimental groups. Total Se intake was greater in supplemented groups, but it was not altered in BD exposed animals. Relative hepatic weight, serum transaminases (AST and ALT) and their ratio joint to total bilirubin serum values were increased in BD group with reference to C ones. The Se supplementation used in BD animals significantly decreased the ratio AST/ALT, mainly by decreasing AST serum values (Table 1). Serum Se levels were decreased in BD rats as compared to C and BDS groups. CS rats had the highest Se serum levels. Serum MDA levels were the highest in BD animals with regard to C and BDS rats. However, BDS rats had higher MDA serum levels than CS ones. Therefore, BD rats had near half the serum Se/MDA ratio value of C rats; Se supplementation to BD rats increased it in 25%. The serum Se/MDA ratio was also increased in CS animals as compared to C ones (Table 1).

Regarding cardiometabolic risk factors, there were no differences in weight, length, BMI or thoracic circumference values among the experimental groups; however, the abdominal thoracic ratio was significantly increased in BD rats with reference to C ones. BD rats also had higher values of TG, gluc and chol than C ones. BDS group had lower TG serum levels than BD rats. Nonetheless, they present higher gluc serum values than CS ones. Whereas albuminuria was not affected by the treatment used in this study, MBP was increased in BD rats and significantly decreased after Se supplementation (Table 2).

Acute ethanol exposed adolescent rats had lower hepatic expression of IRS-1 than control rats ($p<0.001$). Se supplementation to BD rats significantly increased this expression ($p<0.05$), but had lower values than CS rats ($p<0.05$) (Figure 1).

Hepatic AMPK activation (pAMPK expression) was significantly decreased in BD rats, and also the pAMPK/AMPK ratio, as compared to control animals ($p<0.01$). Se supplementation to BD animals significantly increased those parameters ($p<0.05$) (Figure 2).

Repeated BD exposition decreased SIRT1 hepatic expression respecting control rats ($p<0.05$); Se supplementation to BD rats significantly increased SIRT1 expression ($p<0.05$) (Figure 3).

4. DISCUSSION:

4.1. Nutritional and hepatic parameters

From a nutritional point of view, neither BD nor Se supplementation affects Kcal intake or body weight. With regard to liver, previous research in this laboratory has described hepatic damage associated to BD exposition during adolescence[37,41,42]. Moreover, this lab has previously described that Se plays an important role in the liver of BD exposed animals, since BD deeply affects hepatic selenoprotein expression; and when selenite is supplied, hepatic selenoproteins expression increased, leading to a better oxidative, inflammatory and apoptotic liver profile [3]. In that previous study, we used the liver from the same rats than in the current one, and we found that liver GPx activity was decreased in BD animals, and that Se supplementation avoided this situation (C: 98.3 ± 5.2; BD: 76.43 ± 4.64; CS: 131.23 ± 9.34; 118.5 ± 8.9 U/mg protein). Furthermore, we found higher lipid peroxidation in the liver of BD rats, which decreased with Se supplementation (C: 0.087 ± 0.006; BD: 0.14 ± 0.007 ; CS: 0.09 ± 0.006; BDS: 0.10 ± 0.008 mol/mg protein); indicating that there is an important oxidative balance disruption related to OS in the liver of BD rats, avoided when Se is supplemented to those rats.

According to those data, in the present study it has been confirmed that BD leads to hepatomegaly, higher transaminases AST/ALT ratio and higher bilirubin serum levels, being all these markers related to hepatocytes damage. What is more, the serum ratio Se/MDA, suggested as an indicator of hepatic damage caused by alcohol consumption via oxidation, was deeply decreased[43], confirming the deleterious effects of BD on the liver of adolescent rats, and its relation to OS. Se supplementation to BD rats increased Se/MDA serum levels and partially increased AST/ALT ratio, indicating a better hepatic function in these animals.

4.2. Cardiometabolic risk factor

BD is related to hepatic damage; with just a single BD event, mild steatosis could occur, and when BD is repeated, macro-steatosis could manifest[44]. Steatosis is related to fat and glucose metabolism disruption, leading to future cardiovascular disorders. Therefore, different BD models can also lead to high gluc and lipid serum levels, IR and HTA[9–11]; these alterations are factors of MS development. According to that, in this study, when cardiometabolic risk factors were measured, it was found that BD adolescent rats had high serum TG, chol and gluc values; higher abdominal thoracic ratio, which is related to the hepatomegaly previously found; and high blood pressure. On the contrary, albuminuria was not affected. This implies a metabolic deregulation process which affects the metabolism and energy homeostasis. In this context, Se supplementation to these animals decreased the abdominal thoracic ratio, the MBP and TG serum levels; however, chol and gluc serum levels were still increased. Despite

the fact that serum metabolic profile was not improved with Se supplementation, this trace element has important beneficial effects on cardiovascular function. For instance, Se supplementation to BD adolescent rats improves hydric-saline balance[4]; decreases vascular proteins related to angiogenesis; improves myocyte function; and avoids the tachycardia induced by BD[5]. Nevertheless, it is known that Se is deeply related to gluc and lipid metabolism in an U shaped relationship, which needs to be well elucidated[45].

4.3. Insulin resistance

Individuals with a history of BD have an increased risk of developing MS, type 2 diabetes and IR[46]. In fact, Steiner et al.[47] defend that ethanol greatly impact gluc metabolism by different mechanisms, including impairments in intestinal gluc absorption; endogenous pancreatic insulin secretion alterations; gluc effectiveness; and counter-regulatory effects. In the present study, consistent with the hyperglycemia found, BD exposition during adolescence leads to lower expression of hepatic IRS-1, which implies lower insulin hepatic sensitivity and an impairment in gluc homeostasis and insulin action. Alwahsh et al.[48] have found that alcohol induces JNK-1 activation, phosphorylating hepatic IRS-1 and rendering it inactive, while contributing to hepatic IR and probably hyperinsulinemia, since the liver is the mayor site of insulin clearance. If this IR is maintained, in the long run, MS could occur. This is especially worrying during adolescence, because these pathologies will have time enough to develop.

Se and hepatic selenoproteins have also been directly related to the IR process. Low Se deposits are related to OS and IR, since it is necessary for insulin secretion and sensitivity[49]. To the contrary, high GPx1 hepatic expression, by over-quenching intracellular H_2O_2 required for insulin sensitizing, downregulates IRS-1 action and leads to IR[50]. However, since BD exposition leads to a depletion of Se hepatic deposits and GPx1 activity, our Se supplementation therapy acts efficiently by increasing Se deposits, GPx1 activity and IRS-1 expression, and thus, avoiding the IR process in part. Moreover, the dose of selenite supplementation used was correct and safe, since the Se hepatic repletion found in CS rats did not affect IRS-1 expression or gluc serum levels.

4.4. Energetic balance

Taking into account the results obtained, it could be concluded for the first time that BD exposition during adolescence, the main pattern of alcohol consumption among teenagers, affects AMPK and SIRT1 hepatic expression. This downregulation disrupts hepatic energy balance and could be implicated in the development of steatosis, since it affects Acetyl-CoA

carboxylase (ACC) and sterol regulatory element-binding protein 1 (SREBP1) activities[51]. AMPK decreases ACC activity; this enzyme catalyzes the irreversible carboxylation of acetyl-CoA to produce malonyl-CoA, which in turns increases lipogenesis and avoids lipolysis. When ethanol decreased AMPK activity, ACC increased and lipogenesis appeared, leading to the accumulation of fat in the liver. Ethanol, and even more when it is administered in an acute way, increases oxidative phosphorylation in mitochondria, increasing ATP production and ROS generation; both actions decrease AMPK synthesis and phosphorylation (Figure 4).

SIRT1 modulates SREBP1 expression; SREBP1 increases glycolysis and lipogenesis, and it is known that it leads to liver steatosis in the IR process, like in this BD exposition. SIRT1 suppresses SREBP1 activity, and protects against development of fatty liver disease[52]. BD decreases SIRT1 hepatic expression not only by the high $NADH/NAD^+$ ratio created in its oxidative metabolism via alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)[53], but also by the ROS generated by CYP2E1 and the oxidative phosphorylation in mitochondria. This contributes to a higher SREBP1 activity and lipogenesis (Figure 4). Interestingly, the inhibitory effect of SIRT1 on ADH activity has been described, being important in terms of BD tolerance, and pointing to the ratio ADH/SIRT1 as an important regulatory hub for ethanol metabolism and susceptibility[10]. Furthermore, it seems that SIRT1 also plays an important role in the IR process, but it is not still well elucidated[54]. It is known that SIRT1 depletion induced JNK-1 activation; increased serine phosphorylation of IRS-1, along with inhibition of insulin signaling steps, such as tyrosine phosphorylation of IRS-1; and phosphorylation of Akt and ERK. In contrast, treatment of cells with specific small molecule SIRT1 activators led to an increase in glucose uptake and insulin signaling, as well as a decrease in serine phosphorylation of IRS-1 [55]. Similar results, related to SIRT1 and IRS-1/Pi3K/AKT were found by Lin et al.[56] These results are according to our data, since BD leads to a lower SIRT1 and IRS-1 expression and a higher gluc serum levels.

In order to know the role that OS plays in the modulation of these proteins, different antioxidants have been used in ethanol exposed animals to activate AMPK[24,26,57]. In this study, Se increased AMPK activity and SIRT1 hepatic expression. The antioxidant Se is not only related to the hepatic dysfunction found after BD exposition, but also it has been found that can modulate hepatic AMPK activity in MS models[58]. Apart from the action of GPx1 in insulin cascade signaling, the Selenoprotein P (SeP) has been proved to be a hepatokine, which in IR conditions is increased and impairs insulin signaling in the liver by inactivating hepatic AMPK [59]. It is described in adolescent rats exposed to the same BD protocol used in this study, that BD decreases GPx1 expression, but not SelP, and that Se supplementation raises GPx1 to normal

levels without affecting SelP expression[3]. Therefore, the upregulation of AMPK orchestrated by Se supplementation does not seem to be related to SelP expression, which indicates that more studies are needed to understand SelP function in IR. Hence, Se main action is related to its antioxidant activity via different GPxs. This implies that OS plays a pivotal role in AMPK inactivation by BD. Since BDS rats are exposed to the same amount of ethanol than BD ones (ethanol has been administered i.p.) and so the ATP production should be similar, there should be an important indirect action of ROS on AMPK activation after BD exposition. Besides, Se supplementation also increases SIRT1 expression probably by avoiding OS, since the ratio NADH/NAD+ should be similar to BD animals; this protein also activates AMPK, reinforcing the importance of OS in SIRT1 and AMPK inactivation. This link is even more important, since it is increasingly clear that AMPK activation has also multiple actions on inflammatory signaling processes [60], ameliorating vascular endothelial dysfunction [61] and inducing autophagia in the cardiomyocytes [62], becoming a promising target for the treatment of MS and CVD.

In conclusion, the OS specifically generated by BD exposition during adolescence has a pivotal role in metabolic and energetic balance. Se supplementation, by avoiding OS, improves the hepatic expression of pAMPK and SIRT1, contributing to improve metabolic (better lipid profile and IRS-1 expression) and vascular function (lower MBP), and to increase hepatic functionality (lower AST/ALT ratio). All these actions decrease cardiometabolic risk factors development in the short and long term, and could disrupt the relationship among BD and MS, two problems which are currently affecting adolescents.

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5. BIBLIOGRAPHY.

1. Chung T, Creswell KG, Bachrach R, Clark DB, Martin CS. Adolescent Binge Drinking. *Alcohol Res*. 2018;39(1):5-15.

2. Jones SA, Lueras JM, Nagel BJ. Effects of Binge Drinking on the Developing Brain. *Alcohol Res*. 2018;39(1):87-96.

3. Ojeda ML, Carreras O, Sobrino P, Murillo ML, Nogales F. Biological implications of selenium in adolescent rats exposed to binge drinking: Oxidative, immunologic and

409 apoptotic balance. *Toxicol Appl Pharmacol*. 2017;329.

410 4. Sobrino P, Ojeda ML, Nogales F, Murillo ML, Carreras O. Binge drinking affects kidney
411 function, osmotic balance, aldosterone levels, and arterial pressure in adolescent rats:
412 the potential hypotensive effect of selenium mediated by improvements in oxidative
413 balance. *Hypertens Res*. Published online May 9, 2019.

414 5. Ojeda ML, sobrino P, Rua RM, Nogales F, Carreras O. Selenium supplementation
415 improves heart rate and systolic blood pressure in adolescent rats exposed to binge
416 drinking by different mechanisms. *J. Substance Abuse Treatment. 2020.*

417 6. Lu Y, Cederbaum AI. CYP2E1 and oxidative liver injury by alcohol. *Free Radic Biol Med*.
418 2008;44(5):723-738.

419 7. Abdelmegeed MA, Banerjee A, Jang S, et al. CYP2E1 potentiates binge alcohol-induced
420 gut leakiness, steatohepatitis, and apoptosis. *Free Radic Biol Med*. 2013;65:1238-1245.

421 8. Kołota A, Głąbska D, Oczkowski M, Gromadzka-Ostrowska J. Oxidative stress
422 parameters in the liver of growing male rats receiving various alcoholic beverages.
423 *Nutrients*. 2020;12(1).

424 9. Piano MR, Mazzuco A, Kang M, Phillips SA. Cardiovascular Consequences of Binge
425 Drinking: An Integrative Review with Implications for Advocacy, Policy, and Research.
426 *Alcohol Clin Exp Res*. 2017;41(3):487-496.

427 10. Marmier S, Dentin R, Daujat-Chavanieu M, et al. Novel role for carbohydrate responsive
428 element binding protein in the control of ethanol metabolism and susceptibility to
429 binge drinking. *Hepatology*. 2015;62(4):1086-1100.

430 11. Souza-Smith FM, Ford SM, Simon L, Molina PE. Repeated Binge-Like Alcohol
431 Intoxication: Depot-Specific Adipose Tissue Immuno-Metabolic Dysregulation. *Shock*.
432 2017;48(2):243-250.

433 12. Higgins V, Adeli K. Pediatric Metabolic Syndrome: Pathophysiology and Laboratory
434 Assessment. *EJIFCC*. 2017;28(1):25-42.

435 13. Cantó C, Auwerx J. PGC-1α, SIRT1 and AMPK, an energy sensing network that controls
436 energy expenditure. *Curr Opin Lipidol*. 2009;20(2):98-105.

437 14. Ruderman NB, Xu XJ, Nelson L, et al. AMPK and SIRT1: A long-standing partnership? *Am*

438 *J Physiol - Endocrinol Metab*. 2010;298(4).

439 15. Giovannini L, Bianchi S. Role of nutraceutical SIRT1 modulators in AMPK and mTOR
440 pathway: Evidence of a synergistic effect. *Nutrition*. 2017;34:82-96.

441 16. T. R, Y. L, S. Y, et al. Aging aggravates alcoholic liver injury and fibrosis in mice by
442 downregulating Sirtuin1expressionin the liver. *Hepatology*. Published online 2016.

443 17. Huang F, Wang J, Yu F, et al. Protective effect of Meretrix meretrix oligopeptides on
444 high-fat-diet-induced non-alcoholic fatty liver disease in mice. *Mar Drugs*. 2018;16(2).

445 18. Jiang Z, Zhou J, Zhou D, Zhu Z, Sun L, Nanji AA. The adiponectin-SIRT1-AMPK pathway in
446 alcoholic fatty liver disease in the rat. *Alcohol Clin Exp Res*. 2015;39(3):424-433.

447 19. Qiu P, Li X, Kong DS, Li HZ, Niu CC, Pan SH. Herbal SGR Formula Prevents Acute Ethanol-
448 Induced Liver Steatosis via Inhibition of Lipogenesis and Enhancement Fatty Acid
449 Oxidation in Mice. *Evidence-based Complement Altern Med*. 2015;2015.

450 20. Shearn CT, Backos DS, Orlicky DJ, Smathers-McCullough RL, Petersen DR. Identification
451 of 5' AMP-activated kinase as a target of reactive aldehydes during chronic ingestion of
452 high concentrations of ethanol. *J Biol Chem*. 2014;289(22):15449-15462.

453 21. Cederbaum AI. Alcohol Metabolism. *Clin Liver Dis*. 2012;16(4):667-685.

454 22. Ramadori P, Cubero FJ, Liedtke C, Trautwein C, Nevzorova YA. Alcohol and
455 Hepatocellular Carcinoma: Adding Fuel to the Flame. *Cancers (Basel)*. 2017;9(10).

456 23. Pan JH, Lim Y, Kim JH, et al. Root bark of Ulmus davidiana var. japonica restrains acute
457 alcohol-induced hepatic steatosis onset in mice by inhibiting ROS accumulation. *PLoS*
458 *One*. 2017;12(11):1-13.

459 24. Nagappan A, Jung DY, Kim JH, Lee H, Jung MH. Gomisin N alleviates ethanol-induced
460 liver injury through ameliorating lipid metabolism and oxidative stress. *Int J Mol Sci*.
461 2018;19(9).

462 25. Wegner SA, Pollard KA, Kharazia V, et al. Limited Excessive Voluntary Alcohol Drinking
463 Leads to Liver Dysfunction in Mice. *Alcohol Clin Exp Res*. 2017;41(2):345-358.

464 26. Nagappan A, Kim JH, Jung DY, Jung MH. Cryptotanshinone from the salvia miltiorrhiza
465 bunge attenuates ethanol-induced liver injury by activation of AMPK/SIRT1 and Nrf2
466 signaling pathways. *Int J Mol Sci*. 2020;21(1).

27. Zeng T, Zhang C-L, Song F-Y, Zhao X-L, Xie K-Q. Garlic oil alleviated ethanol-induced fat accumulation via modulation of SREBP-1, PPAR-α, and CYP2E1. *Food Chem Toxicol*. 2012;50(3-4):485-491.

28. Price NL, Gomes AP, Ling AJY, et al. SIRT1 is required for AMPK activation and the beneficial effects of resveratrol on mitochondrial function. *Cell Metab*. 2012;15(5):675-690.

29. Brigelius-Flohé R. Introduction to serial reviews on selenium and diabetes type 2—An unexpected link. *Free Radic Biol Med*. 2013;65:1536-1537.

30. Wullaert A, van Loo G, Heyninck K, Beyaert R. Hepatic tumor necrosis factor signaling and nuclear factor-kappaB: effects on liver homeostasis and beyond. *Endocr Rev*. 2007;28(4):365-386.

31. Greten FR, Eckmann L, Greten TF, et al. IKKbeta links inflammation and tumorigenesis in a mouse model of colitis-associated cancer. *Cell*. 2004;118(3):285-296.

32. Ojeda ML, Carreras O, Díaz-Castro J, Murillo ML, Nogales F. High- and low- selenium diets affect endocrine energy balance during early programming. *Toxicol Appl Pharmacol*. 2019;382:114744.

33. Schomburg L. The other view: the trace element selenium as a micronutrient in thyroid disease, diabetes, and beyond. *Hormones*. 2020;19(1):15-24.

34. Guo L, Xiao J, Liu H, Liu H. Selenium nanoparticles alleviate hyperlipidemia and vascular injury in ApoE-deficient mice by regulating cholesterol metabolism and reducing oxidative stress. *Metallomics*. 2020;12(2):204-217.

35. YI HW, ZHU XX, HUANG XL, LAI YZ, TANG Y. Selenium-enriched Bifidobacterium longum protected alcohol and high fat diet induced hepatic injury in mice. *Chin J Nat Med*. 2020;18(3):169-177.

36. Yang JG, Hill KE, Burk RF. Dietary selenium intake controls rat plasma selenoprotein P concentration. *J Nutr*. 1989;119(7):1010-1012.

37. Ojeda ML, Rua RM, Nogales F, Díaz-Castro J, Murillo ML, Carreras O. The Benefits of Administering Folic Acid in Order to Combat the Oxidative Damage Caused by Binge Drinking in Adolescent Rats. *Alcohol Alcohol*. 2016;51(3):235-241.

496 38. Ojeda ML, Nogales F, Vázquez B, Delgado MJ, Murillo ML, Carreras O. Pharmacology
497 and cell metabolism: Alcohol, gestation and breastfeeding: Selenium as an antioxidant
498 therapy. *Alcohol Alcohol*. 2009;44(3).

499 39. Lowry OH, Rosebrough NJ, Farr AL, Randal RJ. Protein measurement with the Folin
500 phenol reagent. *J Biol Chem*. 1951;193(1):265-275.

501 40. Draper HH, Hadley M. Malondialdehyde determination as index of lipid Peroxidation.
502 *Methods Enzymol*. 1990;186(C):421-431.

503 41. Nogales F, Rua RM, Ojeda ML, Murillo ML, Carreras O. Oral or intraperitoneal binge
504 drinking and oxidative balance in adolescent rats. *Chem Res Toxicol*. 2014;27(11).

505 42. Ojeda ML, Rua RM, Murillo ML, Carreras O, Nogales F. Binge drinking during
506 adolescence disrupts se homeostasis and its main hepatic selenoprotein expression.
507 *Alcohol Clin Exp Res*. 2015;39(5).

508 43. Rua RM, Ojeda ML, Nogales F, et al. Serum selenium levels and oxidative balance as
509 differential markers in hepatic damage caused by alcohol. *Life Sci*. 2014;94(2):158-163.

510 44. Ghosh Dastidar S, Warner J, Warner D, McClain C, Kirpich I. Rodent Models of Alcoholic
511 Liver Disease: Role of Binge Ethanol Administration. *Biomolecules*. 2018;8(1):3.

512 45. Stranges S, Rayman MP, Winther KH, Guallar E, Cold S, Pastor-Barriuso R. Effect of
513 selenium supplementation on changes in HbA1c: Results from a multiple-dose,
514 randomized controlled trial. *Diabetes, Obes Metab*. 2019;21(3):541-549.

515 46. Lindtner C, Scherer T, Zielinski E, et al. Binge Drinking Induces Whole-Body Insulin
516 Resistance by Impairing Hypothalamic Insulin Action. 2013;5(170):1-26.

517 47. Steiner JL, Crowell KT, Lang CH. Impact of alcohol on glycemic control and insulin action.
518 *Biomolecules*. 2015;5(4):2223-2246.

519 48. Alwahsh SM, Xu M, Schultze FC, et al. Combination of alcohol and fructose exacerbates
520 metabolic imbalance in terms of hepatic damage, dyslipidemia, and insulin resistance in
521 rats. *PLoS One*. 2014;9(8).

522 49. Xu J, Wang L, Tang J, et al. Pancreatic atrophy caused by dietary selenium deficiency
523 induces hypoinsulinemic hyperglycemia via global down-regulation of selenoprotein
524 encoding genes in broilers. Jadhao SB, ed. *PLoS One*. 2017;12(8):e0182079.

525 50. McClung JP, Roneker CA, Mu W, et al. Development of insulin resistance and obesity in
526 mice overexpressing cellular glutathione peroxidase. *Proc Natl Acad Sci U S A*.
527 2004;101(24):8852-8857.

528 51. Jiang Z, Zhou J, Zhou D, Zhu Z, Sun L, Nanji AA. The adiponectin-SIRT1-AMPK pathway in
529 alcoholic fatty liver disease in the rat. *Alcohol Clin Exp Res*. 2015;39(3):424-433.

530 52. Ponugoti B, Kim DH, Xiao Z, et al. SIRT1 deacetylates and inhibits SREBP-1C activity in
531 regulation of hepatic lipid metabolism. *J Biol Chem*. 2010;285(44):33959-33970.

532 53. Wilson DF, Matschinsky FM. Ethanol metabolism: The good, the bad, and the ugly. *Med*
533 *Hypotheses*. 2020;140.

534 54. Aditya R, Kiran AR, Varma DS, Vemuri R, Gundamaraju R. A Review on SIRtuins in
535 Diabetes. *Curr Pharm Des*. 2017;23(16).

536 55. Yoshizaki T, Schenk S, Imamura T, et al. SIRT1 inhibits inflammatory pathways in
537 macrophages and modulates insulin sensitivity. *Am J Physiol - Endocrinol Metab*.
538 2010;298(3).

539 56. Lin KH, Chiu CH, Kuo WW, et al. The preventive effects of edible folic acid on
540 cardiomyocyte apoptosis and survival in early onset triple-transgenic Alzheimer's
541 disease model mice. *Environ Toxicol*. 2018;33(1):83-92.

542 57. Zeng H, Guo X, Zhou F, et al. Quercetin alleviates ethanol-induced liver steatosis
543 associated with improvement of lipophagy. *Food Chem Toxicol*. 2019;125:21-28.

544 58. Ojeda ML, Carreras O, Díaz-Castro J, Murillo ML, Nogales F. High- and low- selenium
545 diets affect endocrine energy balance during early programming. *Toxicol Appl*
546 *Pharmacol*. Published online September 5, 2019:114744.

547 59. Misu H. Pathophysiological significance of hepatokine overproduction in type 2
548 diabetes. *Diabetol Int*. 2018;9(4):224-233.

549 60. Mancini SJ, Salt IP. Investigating the role of AMPK in inflammation. In: *Methods in*
550 *Molecular Biology*. Vol 1732. Humana Press Inc.; 2018:307-319.

551 61. Lu Q, Li X, Liu J, et al. AMPK is associated with the beneficial effects of antidiabetic
552 agents on cardiovascular diseases. *Biosci Rep*. 2019;39(2):1-15.

553 62. Bonnefont-Rousselot D. Resveratrol and cardiovascular diseases. *Nutrients*. 2016;8(5).

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557 **Table 1. Nutritional and hepatic parameters.**

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	C	BD	CS	BDS
Increased body weight (g/day)	5.45 ± 0.24	5.10 ± 0.31	5.38 ± 0.35	5.30 ± 0.35
Kcal intake (Kcal/day)	53.7 ± 3.2	49.44 ± 2.96	55.67 ± 3.34	48.5 ± 2.9
Se from solid intake (µg/day)	3.1 ± 0.2	2.8 ± 0.2	3.13 ± 0.3	2.72 ± 0.2
Liquid intake (mL/day)	17.52 ± 1.2	14.2 ± 0.7 *	17.64 ± 0.9	15.57 ± 0.8
Se from liquid intake (µg/day)	--	--	2.46 ± 0.21	2.18 ± 0.19
Total Se intake (µg/day)	3.1 ± 0.2	2.8 ± 0.2 aaa	5.6 ± 0.3 ccc	4.9 ± 0.3
HSI (g/g body weight (%))	3.6 ± 0.10	3.9 ± 0.08 *	3.67 ± 0.15	3.71 ± 0.11
AST (U/L)	127 ± 5.2	223 ± 17.1 ***, a	142 ± 4.5	175 ± 12.1
ATL (U/L)	41 ± 1.4	55 ± 2.9 **	42 ± 3.4	57 ± 3.0 ••
AST/ALT ratio	3.15 ± 0.15	4.1 ± 0.19 *, a	3.4 ± 0.20	3.1± 0.27
Total Bilirubin (mg/dL)	0.53 ± 0.034	0.71 ± 0.041 **	0.53 ± 0.036	0.61 ± 0.035
Serum Se levels (µg/L)	213.1 ± 8.3	180.28 ± 6.3 *, aaa	342.1 ± 8.5 ccc	245.1 ± 9.1 •••
Serum MDA levels (mmol/mg protein)	80.5 ± 4.8	139.4 ± 9.9 ***, a	96.4 ± 6.1	109.2 ± 7.5
Serum Se/MDA	100 ± 6.86	45 ± 5.70 ***, a	130± 7.91 c	72 ± 6.37 ••

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The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (one-way ANOVA) followed by the Tukey´s test. The number of animals in each group is 8. HSI: Hepatic somatic index. Groups: C: control group, BD: binge drinking alcohol group, CS: control selenium group, and BDS: binge drinking alcohol selenium group. Signification: BD vs C: *p<0.05, **p<0.01, ***p<0.001; BD vs BDS: ^{a} p<0.05, ^{aaa} p<0.001; C vs CS: ^{C}p<0.05, ^{CCC}p<0.001; BDS vs CS: •p<0.05, ••p<0.01, •••p<0.001.

Table 2. Cardiometabolic risk factors.

	C	BD	CSe	BDSe
Weight (g)	97 ± 4.6	82.8 ± 5.8	92.2± 4.8	80.9± 6.2
Cranium–caudal length (cm)	16.78 ± 0.26	15.82 ± 0.32	16.7 ± 0.37	15.6 ± 0.18
Body Mass Index (BMI) (kg/m^2)	32.33± 2.1	32.34± 1.9	31.8± 2.3	32.9± 1.8
Thoracic Circumference (cm)	8.34 ± 0.23	8.5 ± 0.44	9 ± 0.35	8.7 ± 0.2
Abdominal circumference (cm)	8.5 ± 0.22	9.45 ± 0.32 *	9.43 ± 0.22 c	8.84 ± 0.26
Abdominal/ Thoracic ratio	1.02 ± 0.06	1.11 ± 0.06 *, a	1.04 ± 0.07	1.01 ± 0.04
Glucose (mg/dL)	169.2 ± 7.2	205.7 ± 6.3 *	175.8 ± 10.2	239.1 ± 12 •••
Triglycerides (mg/dL)	65.6 ± 1.2	95.6 ± 4.6 **,a	72.7 ± 3.6	80.3 ± 4.3
Cholesterol (mg/dL)	86.4 ± 2.1	96 ± 2.3 *	86.3 ± 2.3	101.2 ± 3.3 •
Urine albumin (g/dl)	12.73 ± 1.24	12.43 ± 0.96	11.38 ± 0.80	12.68 ± 1.04
MBP (mmHg)	81.9 ± 3.4	101.9 ± 3.6 **, a	82.6 ± 4.1	90.8 ± 2.9

The results are expressed as mean ± SEM and analysed by a multifactorial analysis of variance (one-way ANOVA) followed by the Tukey´s test. The number of animals in each group is 8. Groups: C: control group, BD: binge drinking alcohol group, CS: control selenium group, and BDS: binge drinking alcohol selenium group. Signification: BD vs C: *p<0.05, **p<0.01; BD vs BDS: ^{a} p<0.05; C vs CS: ^{C}p<0.05; BDS vs CS: •p<0.05, •••p<0.001.

FIGURE CAPTIONS.

Figure 1. Expression of IRS-1 in the liver of adolescent rats. Representative western blot of protein (normalized to β-actin). The results are expressed as mean ± SEM and analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey´s test. The number of animals in each group is 8. Groups: C: control group, BD: binge drinking alcohol group, CS: control selenium group, and BDS: binge drinking alcohol selenium group. Signification: BD vs C: $^{***}p<0.001$; BD vs BDS: ^{a} $p<0.05$; BDS vs CS: $^{\bullet}p<0.05$.

Figure 2. Expression of AMPK (A), pAMPK (B) and its ratio (C) in liver of adolescent rats. Representative western blots of proteins (normalized to β-actin) (D). The results are expressed as mean ± SEM and analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey´s test. The number of animals in each group is 8. Groups: C: control group, BD: binge drinking alcohol group, CS: control selenium group, and BDS: binge drinking alcohol selenium group. Signification: BD vs C: $^{**}p<0.01$; BD vs BDS: ^{a} $p<0.05$.

Figure 3. Expression of SIRT-1 in the liver of adolescent rats. Representative western blot of protein (normalized to β-actin). The results are expressed as mean ± SEM and analyzed by a multifactorial analysis of variance (one-way ANOVA) followed by Tukey´s test. The number of animals in each group is 8. Groups: C: control group, BD: binge drinking alcohol group, CS: control selenium group, and BDS: binge drinking alcohol selenium group. Signification: BD vs C: $^{**}p<0.01$; BD vs BDS: ^{a} $p<0.05$.

Figure 4: Oxidative metabolism of ethanol after BD exposition in hepatocytes, and its relationship to SIRT1 and AMPK via EROS and NADH/NAD+. Effects of selenium supplementation. Ethanol is oxidized in hepatocytes, mostly through the enzyme alcohol dehydrogenase (ADH) which in turn produces an increase in cytoplasmic NADH/NAD+. In BD exposition, ADH is saturated (KM = 1.4 mM) and CYP2E1 increase its activity generating great amount of ROS. The great amount of acetaldehyde generated by these enzymes enters the mitochondria and is oxidized to acetate by acetaldehyde dehydrogenase (ALDH), increasing intramitochondrial NADH/NAD+ ratio. Acetate pass to Acetyl CoA which enters in Krebs cycle (KC) and via oxidative phosphorylation (Ox-Phos) produces ATP and ROS. The increase in ATP and ROS decreases the activity of AMP-dependent protein kinase (AMPK). This decrease leads to higher Acetyl-CoA carboxylase (ACC) activity and higher Malony CoA levels which increases lipogenesis and avoids lipolisis. At high ethanol levels, KC is decreased, increasing Malony CoA levels. High ROS and NADH/NAD+ levels also decrease SIRT-1 activity, which leads to an increase in sterol regulatory element-binding protein 1 (SREBP1) and increases lipogenesis. Decreased SIRT-1 leads to a decreasse in insulin signaling pathway (IRS/PI3K/AKT) increasing insulin resistance (IR). Selenite suplementation by increasing Glutathione Peroxidase (GPx) activity avoids ROS generation and lipid oxidation increasing AMPK and SIRT-1 activities, improving hepatic lipid and energetic profile. Solid lines and hatched lines indicate stimulatory and inhibitory actions, respectively.