*Send Orders for Reprints to reprints@benthamscience.net*

*Current Drug Metabolism*, 2021, *22*, 1-0 

**RESEARCH ARTICLE**

# Profiles of Two Glycaemia Modifying Drugs on the Expression of Rat and Human Sulfotransferases

Sangita Maiti Dutta[1,*], Guangping Chen[2] and Smarajit Maiti[3,4]

[1]*Department of Biological Sciences, Midnapore City College, Midnapore, West Bengal, India;* [2]*Department of Physiological Sciences, Oklahoma State University, 264 McElroy Hall, Stillwater, OK74078;* [3]*Cell and Molecular Therapeutics Laboratory, Department of Biochemistry and Biotechnology, Oriental Institute of Science and Technology, Midnapore-721102, West Bengal, India;* [4]*Founder and Secretary, Agricure Biotech Research Society, Epidemiology and Human Health Division, Midnapore-721101, India*

**Abstract:** ***Aims*:** To study the effects of blood glucose regulating compounds on human and rat sulfotransferases (SULTs) expressions.

***Background*:** Phase-II enzymes, sulfotransferases catalyze the sulfuryl-group-transfer to endogenous/exogenous compounds. The alteration of expressions of SULTs may have influence on the sulfation of its substrate and other biomolecules.

***Objectives*:** The influence of the altered biotransformation might alter different biochemical events, drug-drug interactions and bioaccumulation or excretion pattern of certain drug.

***Methods*:** In this brief study, diabetes-inducing drug streptozotocin (STZ; 10 or 50 mg/kg to male Sprague Dawley rat for 2 weeks) or hyperglycemia controlling drug tolbutamide (TLB 0.1 or 10μM to human hepato-carcinoma cells, HepG2 for 10 days) was applied and the SULTs expressions were verified. Extensive protein-protein (STa, SULT2A1/DHEAST) interactions were studied by the STRING (Search-Tool-for-the-Retrieval-of-Interacting Genes/Proteins) Bioinformatics-software.

***Results*:** Present result suggests that while STZ increased the STa (in rat) (dehydroepiandrosterone catalyzing SULT; DHEAST in human HepG2), tolbutamide decreased PPST (phenol catalyzing SULT) and DHEAST activity in human HepG2 cells. Moderate decreases of MPST (monoamine catalyzing SULT) and EST (estrogen catalyzing) activities are noticed in this case. STa/DHEAST was found to be highly interactive to SHBG/-sex-hormone-binding-globulin; PPARα/lipid-metabolism-regulator; FABP1/fatty-acid-binding-protein.

***Conclusions*:** Streptozotocin and tolbutamide, these two glycaemia-modifying drugs demonstrated regulation of rat and human SULTs activities. The reciprocal nature of these two drugs on SULTs expression may be associated with their contrasting abilities in influencing glucose-homeostasis. Possible association of certain SULT-isoform with hepatic fat-regulations may indicate an unfocused link between calorie-metabolism and the glycemic-state of an individual. Explorations of this work may uncover the role of sulfation metabolism of specific biomolecule on cellular glycemic regulation.

**ARTICLE HISTORY**

Received: July 15, 2020
Revised: October 14, 2020
Accepted: November 03, 2020

*DOI:*
*10.2174/1389200221666201130123837*

**Keywords:** Diabetes, streptozotocin, tolbutamide, sulfotransferase, rat liver, Hep G2.

## 1. INTRODUCTION

Sulfotransferases (SULTs) are important for their ability to modify *via* sulfuryl group transfer to several drugs and biomolecules [1]. A large number of prescription drugs undergo Phase II metabolism and form their different derivatives. Sulfuryl group transfer to a drug increase the polarity of that drug. Increased polarity results in increased solubility of that drug in blood and other body fluids. As a result, the substance is circulated with higher rate in the blood and may be procured by the target cells by their carrier, transporter, receptor or channel proteins. But this is also true that increased polarity/solubility will increase the rate of excretion and the clearance of the substance. As a result, after a certain time the availability of that drug declines [1, 2]. During this process bioactivation of some of those drugs is possible [2]. This fact justifies an extensive research on drug metabolism and especially those drugs which are used in different types of disorders, metabolic-syndrome like cancers, diabetes and vascular diseases [3]. A large number of individuals consume these drugs for years to decades and sometime throughout the life. How the sulfation metabolism of the drug modifies the chemistry of that drug and how that modification impact on physiological or pathological mechanism is worth verifying [4]. On the other hand, sulfation metabolism of that drug or even other bio-molecules may have impact on disease mechanisms and/or its therapeutic pathways [5]. So, the studies drug-mediated alterations in SULTs expression may focus insight of the disease pathogenesis [6].

Endogenous substances like steroids are the natural physiological molecules having general as well as gender specific functions. Testosterone, dehydroepiandrosterone (DHEA) is male and estrogen and progesterone are female specific steroids. These endogenous molecules influence reproductive function. These molecules are readily catabolized during sulfation metabolism by mainly DHEAST/SULT2A1 and EST or SULT1E1 [7]. Some therapeutic agents are also added by the polar sulfuryl group. In this regard, an-

* Address correspondence to this author at the Department of Biochemistry and Biotechnology, Cell & Molecular Therapeutics Lab, OIST, Midnapore-721102; Tel: 9474504269; E-mail: maitism@rediffmail.com

1389-2002/21 $65.00+.00 

ticancer drug doxorubicin is an important example in this category [8]. Endogenous bio-molecules like monoamines, steroids and bile acids are also metabolized by sulfation reactions and control the physiological processes [9, 10]. Our previous report suggests that anticancer drug tamoxifen, MTx, rhein and emodine can induce rat sulfotransferases [11]. The ethyl alcohol and some vitamins like A and folate were also demonstrated to induce sulfotransferase expression [12, 13]. Several of the endogenous molecules like steroids and monoamines are related to glucose homeostasis of the body, so their sulfation might have some direct or indirect roles on the glycemic state of the body [14]. Our present study may help for the understanding the disease mechanism related to SULT mediated sulfation metabolism.

In the current study, two contrasting drugs (judging by their impact on glycaemia) were taken, which have impact on glucose homeostasis. Streptozotocin (STZ) is a pharmacological agent employed to create a diabetic condition in animal experimental model and tolbutamide is used to control the hyperglycemic state. These drugs were tested to study their impact on the expression of rat and human SULTs isoforms. Present study and its further extrapolation may unravel how the SULTs expression can be correlated to the contrasting glycemic potentials of these two drugs. This may further add any new insight on sulfation-mediated influence of the hyperglycemic mechanism.

## 2. MATERIALS AND METHODS

### 2.1. Chemicals

Tolbutamide, Streptozotocin, b-naphthol, (14C) b-naphthol (4.7 mCi/mmol), p-nitro-phenyl sulfate (PNPS), 30-phosphoadenosine-50-phosphosulfate (PAPS), and [1,2,6,7-3H(N)] dehydroepiandrosterone ([3H]DHEA, 60 Ci/ mmol) were purchased from Sigma-Aldrich (St. Louis, MO). SDS-polyacrylamide gel electrophoresis reagents were obtained from Bio-Rad (Hercules, CA). Western blot chemiluminescence reagent kits (Super Signal West Pico Stable Peroxide and Super Signal West Pico Luminol/ Enhancer solutions) were purchased from Pierce Chemical (Rockford, IL). Nitrocellulose membrane (Immobilon-P; Millipore Corporation, Bedford, MA) used during Western blot procedure was purchased from Fisher Scientific (Fair Lawn, NJ). Total RNA extraction kit (RNeasy mini protection kit) was supplied by Qiagen (Valenica, CA). One-step RT-PCR kit was purchased from Promega (Madison, WI). Antibodies against ASTIV [10] and STa [11] were provided by Dr. Michael W. Duffel (Division of Medicinal and Natural Products Chemistry, College of Pharmacy, The University of Iowa, Iowa City, IA). Protein assay reagent was purchased from Bio-Rad. All other reagents and chemicals were of the highest analytical grade available.

### 2.2. Animals and Drug Treatment

Male and female Sprague-Dawley rats (Harlan, Indianapolis, IN) 10- to 11-weeks old and 200-300 g body weight were used in this investigation. Rats were housed in a temperature- and humidity-controlled room and supplied with rodent chow and water for at least 1 week before use. For all animal experiments, proper permissions were obtained from the Institutional (Oriental Institute of Science and Technology, OIST/EC/Animal/2018/012) Internal Review Board [IRB]. We followed all ethical norms and maintained requisite regulatory affairs. All the procedures were done in accordance with the Helsinki Declaration (2000) and National Institutes of Health (NIH) guidelines. Rats were divided into 4 groups with 6 in each. STZ solution freshly prepared (in sterile distilled water) was administered by gavages with 0, 10 or 50 mg/kg/day for 2 weeks. Primarily, STZ was tested in rat to generate a dose response curve and toxicity study was conducted to select the applicable dose. Serum ALP, SGOT, SGPT and oxidative stress marker malondialdehyde were tested. It is noticed that present STZ dose did not develop any cytotoxicity but responded well in terms of present objectivity. The present dose was applied by several other investigators [15, 16]. The animals were sacrificed (i.p. pentobarbital 60 mg/kg) [15,16] 24h after the final drug treatment. And those were fasted overnight before the day of sacrifice. Livers were collected, washed with sterile, ice-cold NaCl (0.9%, w/v) solution, and kept in dry ice bath. All the tissues were stored at -80ºC until use.

### 2.3. Cell Culture and Drug Treatment

The HepG2 cells were obtained from the American Type Culture Collection (Manassas, VA). HepG2 Cells were grown and maintained in Dulbecco's Modified Eagles's Medium Nutrient Mixture F-12 Ham (Sigma) supplemented with L-glutamine and 15mMHEPES, and 10% fetal bovine serum (FBS). For endogenous study in Hep G2 cells, cells were seeded in 10-cm plates at a density of $5\times10^6$cells per plate. On day 1, tolbutamide was added to the medium. The medium was refreshed every 2 days with the addition of new drug. After treatment for the required time, the cells were harvested [12].

The cultures were incubated at 37°C in a humidified incubator containing 5% $CO_2$, 95% air [13]. After seeding at 0 days, on day 1, TLB (0, 0.1, 1, 10 μM final) was added (solution with ethanol under warm heating) to the medium in properly marked plates. HepG2 cells were initially tested with different concentration of tolbutamide application to investigate the dose and duration response nature. At the same time, cells were tested for toxicity, oxidative stress study by checking ALP, SGOT, SGPT and malondialdehyde assay. We found that in the current study that 15 to 20 μM dose increased the ALP and malondialdehyde level which were the marker of cytotoxicity and oxidative stress, respectively. But at 10 μM tolbutamide dosage there was almost no cytotoxicity. Control (0) plates were added to the vehicle. The medium was refreshed every 3 days with the new addition of corresponding drug. On day ten the cells were harvested. Cytosols were prepared from the cultured cells. The cell pellet was dissolved and homogenized in 1000μL lysis buffer (150 mM NaCl,20 mM Tris, 1 mM EDTA, 1 mM DTT, 0.1 mg/mL trypsin inhibitor, 0.3% Tween-20 (V/V), 1 mM PMSF). Debris was removed by centrifugation at 13,000 rpm for 10 min, and the supernatant was used for biochemical and blotting methods.

### 2.4. Cytosolic Sample Preparation

Liver homogenates and Hep G2 cells were prepared with 50mM Tris buffer containing 0.25M sucrose, pH 7.5 containing 0.01 mg/ml trypsin inhibitor and 10 μg/ml phenylmethylsulfonyl fluoride (PMSF). All homogenates were centrifuged at 100,000g for 1 h at 4ºC Cytosol aliquots were collected and preserved at 80°C for enzymatic assay and Western blot [10, 13].

### 2.5. Enzyme assays

Two different enzyme assay methods were used.

#### *2.5.1. PNPS Assay*

*β*-naphthol sulfation activity from liver cytosol was determined as previously described [11, 13]. This assay determines phenol sulfation activities of different isoforms of phenol sulfating STs. Briefly, sulfation activity was determined in a reaction mixture containing 50mM Tris buffer, pH 6.2, 5mM PNPS, 20 μM PAPS, and

0.1mM b-naphthol. Rat liver cytosols (50 µg protein) were used as the enzyme source in a total reaction volume of 250 µl. After 30 min incubation at 37 C in a shaking water bath, the reaction was stopped by adding 250 µl of 0.25M Tris, pH 8.7 The reaction mixtures were read at 401nm in a spectrophotometer [12]. Specific activity (SA) was expressed as nanomole per minute per milligram of protein. The data shown in the figures were the average data sets collected from 6 different animals.

#### *2.5.2. Radioactive Assay*

Dehydroepiandrosterone (DHEA) sulfation activities in liver cytosols were determined by the radioactive assay method previously described [11, 12]. Other ingredients and reaction conditions were same as the PNPS assay mentioned above. To determine DHEA sulfation activity in liver [3H] DHEA (diluted to 0.4 Ci µ/mmol; 2 µM final concentration) was used as substrate. For all assays, 20 µM PAPS was used. Liver cytosol protein (50 µg) was used as enzyme source in a total reaction volume of 250 µl. After 30 min incubation at 37ºC in a shaking water bath, the reaction was stopped by adding 250 µl of 0.25M Tris, pH 8.7 Extraction was performed twice by addition of 0.5 ml of water-saturated chloroform. After the final extraction, 100 µl of aqueous phase was used for scintillation counting. The data shown in the figures were the average of 3 independent data sets collected from 3 different animals. PAPS was eliminated from the controls of both assay methods. Assays were run in duplicate and the average of the results was used for enzyme activity calculations.

Estrogen sulfotransferase Enzyme Activity Assay: hSULT1E1 (EST) activities in Hep G2 cell cytosol (50µg protein from each protein) sample was determined by the radioactive assay method [12]. [3H]E2 (0.9 Ci/mmol; 0.15µM final concentration) was used as substrate for the reaction. For all assays, 20µM PAPS was used in a total of 250µl reaction mixture containing 50 mM Tris buffer (pH 6.2). After 30-min of incubation in shaking water bath (37°C), the reaction was stopped by adding 250µl of 0.25 M Tris (pH 8.7). Extraction was performed twice by adding 0.5 ml of water-saturated chloroform each time. After the final extraction, aqueous phase was used for scintillation counting.

### 2.6. Western Blot Analysis

Cytosol protein from liver (10 µg) was used in a 10% polyacrylamide gel in an electrophoresis system (Novex, San Diego, CA). After running at 200 V, the protein bands were transferred overnight at 40V onto a nitrocellulose membrane. For rat liver cytosols, membranes were incubated with either rabbit anti-rat AST-IV or rabbit anti-rat STa (1:5000) in TBST (50mM Tris, pH 7.5, 150mM NaCl, and 0.05% (v/v) Tween 20) containing 5% (w/v) dried milk for 2 h on a shaker at room temperature. After incubation, all membranes were washed with TBST for 4x15 min and incubated in secondary antibody (horseradish peroxidase- conjugated Immuno-Pure goat anti-rabbit IgG; H+ L) at 1:5000 dilutions in the same buffer for 2 h. The membranes were washed with TBST for 4x15 min and then with phosphate-buffered saline (PBS) 3x5 min. Fluorescent bands were developed with 1ml of substrate containing the same volume of each Super Signal West Pico Luminol Enhancer solution and Super Signal West Pico Stable Peroxidase solution at room temperature for 5 min. The X-ray films were exposed to the membrane and then developed. Films were scanned and the densitometry analysis was performed in a Gel Documentation and Analysis System from Advanced American Biotechnology and with AAB software (Fullerton, CA) [11-13].

### 2.7. Extraction of Total RNA and RT-PCR

Total RNA was extracted from liver using RNeasy mini protection kit from Qiagen according to supplier's guidelines. The concentration and purity of the extracted RNA were checked spectrophotometrically by measuring 260/280 absorption ratios. The primer pair for AST-IV was designed in our laboratory using the Gene Fisher primer designing and Multi-alignment software. Using the forward primer (FP) 50-GTGTCCTATGGGTCGTGGTA-30 and reverse primer (RP) 50-TTCTGGGCTACAGTGAAGGTA-30 (GenBank Accession No.: X52883), the 299-bp AST-IV cDNA was synthesized [11,13]. The 264-bp STa cDNA was synthesized using the primer pair FP 50-TCCTCAAAGGATATGTTCCG-30 and RP 50-CAGTTCCTTCTCCATGAGAT-30 (GenBank Accession No.: M33329) [11,13]. The nucleotide sequences X52883 and M33329 (GenBank Accession No.) were used as reference sequence for the synthesis of AST-IV and STa cDNA, respectively. The specificity of all primers was tested using the BLAST of the National Center for Biotechnology Information Open Reading Frame software. cDNA synthesis from 1 µg of liver total RNA was performed in a 50µl reaction mixture. The concentrations of the different ingredients were used following supplier's protocol. For control, 500-bp cDNA of rat b-actin was synthesized from the same amount of RNA from respective tissues. The primer pair (FP 50-GATGTACGTAGCCATCCA-30 and RP 50-GTGCCAACCA-GACAGCA-30) for the synthesis of rat $\beta$-actin cDNA was designed in our laboratory using the same software mentioned above [11,13].

### 2.8. Possible Interactions of SULT2A1, Steroid Sulfotransferase with other Proteins

STRING (*Search Tool for the Retrieval of Interacting Genes/Proteins*) is a biological database and web resource that is employed for the prediction of protein-protein interactions [17,18]. In a certain metabolic environment the interactive proteome body is analyzed in relation to their functional networking by this software. String is a pre computed database derived from experimental data, literature mining, analysis of co-expressed genes *etc*. String applies a unique scoring method based on the different types of associations against a common reference set and produces a single confidence score per prediction [19]. Evidence based interactive or interrelated pattern has been deduced by this online resource/analytical software (http://string-db.org/).

### 2.9. Experimental

Student's t test was performed to calculate the statistical significance with the difference between means control and drug treated rats. Data presented in the figures denote means ± SEM of the results collected separately from six different animals or three different experimental cells of human Hep G2.

## 3. RESULTS

By the year 2040, there will be 642 million people worldwide suffering from diabetes mellitus [20]. Long-term therapy with a number or drugs may temporarily control glycemic status but that may result in different vascular disorders and other physiological complications [21]. At the time of diabetic onset, expressions of several drug-metabolizing enzymes (*i.e.* cytochrome P450 (CYP 450) and sulfotransferase (SULTs) are significantly altered [21]. So regulations of the enzymes are important in relation to the disease pathology and usage of the specific drugs.

In the present investigation, treatment with STZ for 2 weeks significantly suppressed aryl sulfotransferase (AST-IV, $p<0.05$ at higher dose) activity, increased hydroxy-steroid sulfotransferase (S-

Ta) enzyme activity ($p$<0.05 and 0.01) (Fig. **1**) paralleling with protein expression as demonstrated in Western blot results (Fig. **2**). (Fig. **3**) of RTPCR results basically demonstrate a variable gene expression pattern of ASTIV but more consistent expression (both at protein and gene levels) of STa or DHEAST/SULT2A1 is presented in rat liver tissues. Our present bioinformatics studies with STRING show that DHEAST is highly interactive and a major of the proteins are related to fat metabolism. SHBG and PPARα are this kind of proteins. Fat metabolism is associated with the major utilization of the carbon skeleton present in the nutrient molecules. And there is a closer association of carbohydrate and fat metabolism. In contrast, TLB treatment at 10 μM of its dosage significantly suppressed human phenol ST (PPST) activity. The dehydroepiandrosterone sulfotransferase (DHEAST) activity decreased with 1μM and 10 μM of TLB treatment in human cells (hepatic carcinoma HepG2 cells). The corresponding substrates (2-napthol and DHEA) sulfation activity supports the protein expression results also. Whether the sulfation metabolism of any bio-molecules is linked to the glucose homeostasis is of great importance for the understanding of the events in hyperglycemia. In this regard, metabolism of glucose homeostasis-interfering drugs and these drugs-induced changes in the expressions of phase II metabolizing enzymes *i.e.* SULTs are absolutely important. A brief report on SULTs regulations by STZ and TLB is presented in this study.

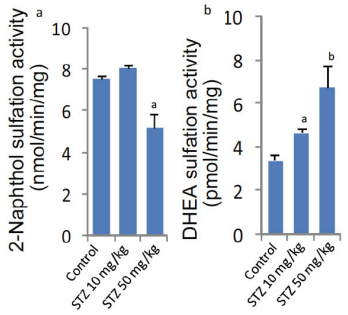


**Fig. (1).** Effects of Streptozotocin on the activities and expression of rat AST-IV and STa. Statistical analysis shows Students's t test and level of significance of differences of treated group versus control group; a p<0.05, b p<0.01, c p<0.001. The AST-IV activity decreased slightly ($p$<0.05) at higher dose of STZ (a).The STa activity significantly and gradually increased at the enzyme activity level (a$p$<0.05 to b$p$<0.01). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

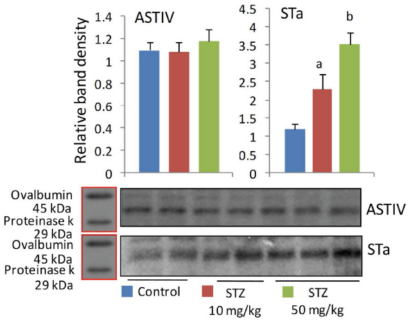


**Fig. (2).** Effects of Streptozotocin on the expressions (Western Blot) of rat AST-IV and STa proteins. And their band density data are also presented as a bar-diagram. Statistical analysis shows Students's t test and level of significance of differences of treated group versus control group; a p<0.05, b p<0.01. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

## 4. DISCUSSION

Some previous report suggests that cytochrome P450 (CYP) tolbutamide 4-hydroxylation (CYP2C9) is regulated by some solvents like acetonitrile, dimethyl sulfoxide (DMSO), and methanol (MeOH) in human hepatocytes suggesting some modification events of tolbutamide [22,23]. This is important for this drug to increase its bioavailability. Intracellular lower availability of glucose and the hyperglycemic state is not tissue or organ specific. So, the higher bioavailability of any anti-diabetic drug significantly increases its efficacy [24]. In case of laboratory experiment, selection of organic solvents is of practical importance because variations of the solvent may change the activities of CYP2C9 by 40%, and CYP2E1 by 11% in human hepatocytes [24]. Present STRING [bioinformatics] data suggests that SULT2A1 is interacting with different isoform of cytochrome p450 group of enzymes, and CYP1A1 is important which has overlapping substrate sharing with different degrees of specificity (Fig. **3**). This suggests strong correlations between different phases of drug metabolizing enzymes. Different isoforms of cytochrome P450 enzymes like CYP1A1 and CYP1B1 are linked to SULT2A1 which is a steroid metabolizing sulfotransferase (Fig. **3**). Moreover, the association between SULTs expression and diabetic/energy regulations is indicative from the results of SULT2A1 relations with PPARα (ligand activated transcription regulator of fat metabolism) and FABP1 (fatty-acid binding protein, helps lipoprotein-mediated cholesterol uptake in hepatocytes) (Fig. **3**). SULT2B1b expression has been shown to regulate lipid metabolism by modulating liver X receptor α, acetyl-CoA carboxylase-1, and fatty acid synthase [25].

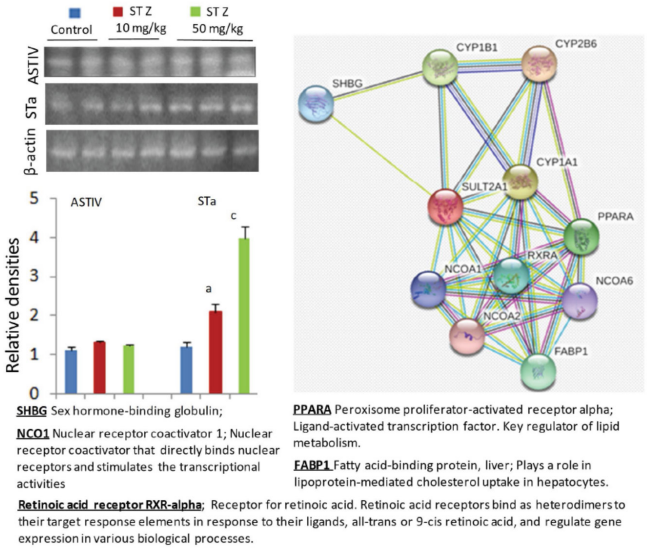


**Fig. (3).** The mRNA expression (RT-PCR level) with the gene specific primers of ASTIV and STa agrees with the Western blot and enzyme activity results. RT-PCR band density analysis is also presented. Statistical analysis shows Students's t test and level of significances of differences of treated group versus control group; (a$p$<0.05 to b$p$<0.01). Right panel of the picture demonstrates the STRING-generated interactive proteome where SULT2A1 is found to be involved in energy metabolism. Nuclear receptor co-activator 1, 2, 3; lipid metabolism regulator, Peroxisome proliferator-activated receptor PPARα, Fatty acid-binding protein, liver Retinoic acid receptor RXR-alpha; Receptor for retinoic acid. Retinoic acid receptors bind as heterodimers to their target response elements in response to their ligands, all-trans or 9-cis retinoic acid, and regulate gene expression in various biological processes. All these interactions suggest calorie metabolism and sugar homeostasis. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

It is pointed out that the changes in steroid levels in response to Peroxisome-proliferators (PP) exposure are caused due to alterations in the levels of P450 enzymes that hydroxylate testosterone and estrogen. This suggests that lipid-transport regulators can also regulate important steroid levels both male and female [26]. Further, the PPAR-α agonist and insulin like growth factor (IGF-1) may regulate SULT1E1 (estrogen-sulfating and inactivating) mRNA expression and influence the levels of active estrogens in endothelial cells and smooth muscle cells. These are the main target of vascular pathologies in diabetic complications [27]. Estrogen has been shown to have an inter-relation with insulin action [28]. So, SULT-regulating drugs like STZ and tolbutamide may have some implications in the maintenance of the glycemic state of an individual. Further studies are necessary in this regard.

Comparison of phase I and phase II enzyme activities in the sub-cellular fractions of cultured cells of several higher-primates (humans, cynomolgus monkeys, rhesus monkeys) suggests that UDP-glucuronosyl transferase, acetaminophen sulfotransferase, and tolbutamide 4-hydroxylase activities are greater in rhesus monkeys than that of human [29]. Those activities were found to be significantly less in the lower mammals (*i.e.* dog). Inversely, lower activity of tolbutamide methylhydroxylase was observed in intestine of human and monkey but not in dog intestines and human Caco-2 cells. Higher intestinal glucuronyl-transferase, N-acetyltransferase, sulfotransferase, and glutathione S-transferase were noticed in human [30]. This suggests that tolbutamide specific drug metabolizing enzymes including sulfotransferase are available in the system. Moreover, expression of drug metabolizing enzymes is species specific depending on their specific pattern of sugar metabolism. This data may have some great practical value to clarify why human experience more incidences of metabolic disorder related to calorie-homeostasis issues.

There is no report on the direct sulfation reaction with streptozotocin and tolbutamide. Tolbutamide is a sulfonylurea (presence of $=SO^2$ group). Sulfation mediated biotransformation of these drugs is not a normal biochemical phenomenon. But, from our current study (and some previous studies in case of streptozotocin) it is clear that both drugs can variably induce different isoforms of rat and human SULTs [31, 32]. These drug-induced SULTs expressions might have significant role in the sulfation of some endogenous molecules (like bioamines, steroids and bile acids) or exogenous molecules like other prescription drugs. Increased sulfation of endogenous and exogenous molecules may have some physiological implications [31,32]. STZ induction of glucocorticoid (cortisol) sulfotransferase, hepatic bile acid sulfotransferase has been reported. Not only sulfation associated changes but also post-sulfation modification of heparan sulfate has been impaired by the STZ-induced inactivation of glucosaminyl N-deacetylase in rat hepatocytes [33].

Report revealed that activity of hepatic UDP-glucuronyltransferase and β-glucuronidase were significantly suppressed by the pretreatment of STZ. Reversal of the STZ-inhibitory effect of only UDP-glucuronyltransferase was demonstrated by the application of insulin [34]. This suggests some association between oppositely acting STZ-insulin. Similarly, regulations of SULTs by STZ and tolbutamide as noticed in the current study may have some physiological significance [35]. Further studies are necessary in this regard.

It may be suggested that metabolism of endogenous molecules by the process of sulfation and other reactions have some relevance in the initiation and pathogenesis of certain diseases. In an extrapolation of this thought it can be said that, drugs used against these diseases should be focused for their possible role on SULTs expressions. Liver is the major metabolic destination in the body which expresses all isoforms of SULTs to participate any type of sulfation metabolism. On the other hand, role of liver in glucose metabolism has been evident. Glucose induced nitric oxide synthase (NOS) expression in liver and NO production regulate insulin sensitivity following its synthesis in the hepatocytes [36]. Our earlier report suggests that estrogen has a great role on insulin mechanism and glucose tolerance factors [37]. Estrogen is important biomolecules having a range of physiological activities starting from the early embryogenesis, morphogenesis and organogenesis. In adolescent period and in adult female it influences several morphological and reproductive functions and even in the male, this hormone has specific biological effects. Report reveals that E2 attenuates diabetic complications in kidney disorder and even it protects kidney electrolytes-transporter and related proteins in normal individuals [38].

Men with type 2 diabetes mellitus are prone to develop carotid atherosclerosis (CA) and serum estradiol concentration has been shown to be inversely correlated to the progression of diabetic CA [39]. High glucose is stress inducing and can develop oxidative stress resulting in the increase of its marker malondialdehyde. Estradiol has been shown to increase GLUT-4 response and insulin sensitivity in type-2 diabetes. Moreover resveratrol, a non-flavonoid antioxidant polyphenol and structurally/functionally similar to estrogen can protect from diabetes by same biochemical pathways of increasing insulin receptor substrate 1 [IRS-1]. But it decreases the expression of p-ERK expression in skeletal muscle and myocardial tissue [40]. This obvious protection against STZ-induced diabetes in overectomized mice is attributed by the antioxidant activities of resveratrol on islet function promotes muscle glucose uptake [40]. In several of our previous report we have shown the protective role of estradiol in diabetes and diabetic complications in cardiovascular pathology [37]. So, the level of estradiol has been important which can be regulated by the expressions and activities of estrogen sulfotransferase (EST or SULT1E1). Estrogen sulfotransferase (SULT1E1) catalyzes the E2 sulfation and due to its inactivation E2 level may increase in the liver tissue [41]. In summary, liver can self-regulate its insulin function by its own E2. Upon SULT1E1-mediated sulfation, E2S is prevented for the binding to estrogen receptor (ER). In summary, drug mediated possible interference in SULT1E1 regulation may have impact on glucose homeostasis.

Other SULT isoform, specifically, SULT1A1 and SULT1A3 activities were lower in disease (fatty liver) states relative to non fatty tissues. Alcoholic cirrhotic liver tissues further contained lower SULT1A1 and 1A3 activities [6]. This suggests that liver is one of the major sites for glucose homeostasis and that may influenced by the sulfation metabolism of some molecules. Present bioinformatics data in (Fig. **3**) validate our point that the alteration of DHEAST/SULT2A1 has a wider interactive role in the regulations of fat metabolism factors like fatty acid binding protein1, FABP1 and PPARα; which are the important regulators of the fat metabolism in liver. So, liver fat metabolism would be influenced in either condition of activation or deactivation of DHEAST. Report reveals that several disease conditions may induce SULT1E1 expressions [42]. SULT1E1 can affect the outcome of the disease, the enzymes sulfotransferase and/or sulfatases (STS) can be potential therapeutic targets in certain diseases [43]. The SULT1E1 expression has been shown detectable in the subcutaneous adipose tissue in obese women and men. And that enzyme is markedly induced in the livers of rodent models of obesity and type-2 diabetes [44]. In the current study, alterations in the regulations of steroid sulfotransferase (Fig. **1** to **5**) might have some influence on lipid metabolism. And that may be the stored fat in liver or in adipocytes. Drastic increase of blood cholesterol and other lipid levels in chronic diabetic patients may justify the fact of altered sulfa-

tion-metabolism of liver fat. Adipogenesis has also been shown to be differentially regulated by SULT1E1 in human adipocytes [43].

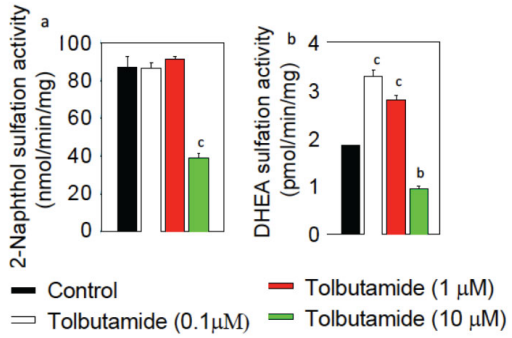


**Fig. (4).** Effect of hyperglycemia controlling drug tolbutamide on human $HepG_2$cells. The enzyme activities are shown in a. PPST and b. DHEAST. Statistical analysis shows Students's t test and level of significance; b $p<0.01$, c $p<0.001$. (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

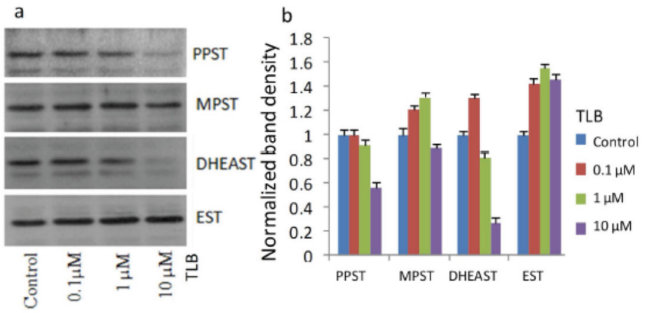


**Fig. (5).** The PPST and DHEAST protein expressions **(Western blot data)** were noticed to decrease dose dependently (a). And this is supported by the densitometry analysis (b). The MPST and EST protein expression was found to increase slightly (10-30%) in dose responsive manner (a). These are supported by the densitometry analysis (b). (*A higher resolution / colour version of this figure is available in the electronic copy of the article*).

The possible interferences of altered enzymatic activity and transporter action might affect the disposition of frequently prescribed glucose-lowering medicines [21]. It is reported that, metformin, is excreted through renal clearance. And the rosiglitazone is metabolized by the hepatic cytochrome P4502C. In contrast, glibenclamide is metabolized by the hepatic CYP3A, canagliflozin is metabolized mainly by UDP-glucuronosyltransferases (UGT) and troglitazone is metabolized by certain isoform of sulfotransferase and UGT [45]. This suggests that different SULTs are responsible for the sulfation of different bio-molecules which are related to hepatic glucose metabolism and hyperglycemia condition. Report reveals that reversal of STZ-induced diabetes with insulin significantly reduced the level of STa mRNA suppression associated with STZ treatment. Moreover, negative transcriptional regulation may be responsible for the changes in STa gene expression that results in the development of STZ-induced diabetes [46]. As we discussed on liver function, it may be relevant to mention that bile acid (BA) hydroxylation (Cyp3a11) and BA sulfation (SULT2A1) takes place with higher rate in the liver of normal mice compared to that of HFD (high fat diet fed) mice. The BA homeostasis in diabetes is not only related to the metabolic syndrome but also result in cognitive dysfunction in the mouse [47]. Metformin may help to improve the glucose and lipid metabolism by influencing the level of serum total bile acids. A combination of high-fat diet HFD and metformin could be effective in the treatment of T2DM [48].

Metabolic regulations of sulfuryl group elimination have been noticed in streptozotocin induced diabetic rats. In this case, billiary excretion of p-nitrophenyl sulphate [32] may be explained as some adaptation by sulfation metabolism. It is noticed as the STZ induction of SULTs expression in the current study. This is also supported by the fact of STZ-induced bile-sulfation enhancement in experimental rat model [31]. Regulations of energy homeostasis are the feature of diabetic condition. Report reveals that long term diabetic condition (T2DM) result in hypercholesterolemia [49]. Cholesterol and oxysterols sulfation and elimination (cholesterol sulfate and oxysterol sulfates) may be increased by the enhancement of the cholesterol sulfotransferase (SULT2B1b) activity. This is accomplished by the hepatocyte nuclear factor 4α (HNF4α) mediated gene regulations [50]. Hepatic gluconeogenesis, a key regulator of energy metabolism may be controlled by HNF4α-SULT2B1b axis [50].

Metabolic dysfunction in adipocytes is also related to the pro-inflammatory state and the TNF-α expression. Moreover, increased SULT1E1 response impairs E2 function and that in turn results in TNF-α responsive inhibition of insulin signaling. This has been differentially demonstrated by the male and female obese mouse model with pancreatic islets [51]. Metabolic role of small molecule like leptin has been shown to influence in the mediation of constitutive androstane receptor (CAR) in type 2 diabetes. Activation of CAR significantly reduces serum glucose levels [52].

Present bioinformatics data indicate a correlation between the functions of phase I (CYP) and phase II SULT2A1 enzymes. Polarization of some drugs requires the orchestrated functions of both phase I and II enzymes. For their own metabolism, most of the drugs require the action of nuclear orphan receptors in association with retinoid xenobiotic receptors (RXR) to induce phase I and II gene expressions. Current STRING data also demonstrate the SULT2A1 and RXRα relationship in association with CAR and VDR. This has been shown in our previous study [53, 54]. Induction of steroid metabolizing enzymes by glucose manipulating drugs also has been shown to be involved with fatty acid binding proteins and lipid metabolism regulator PPARα. These findings suggest the role of SULT2A1 regulations on nuclear receptor mediated nutrient metabolism and energy handling in the body.

Another study supports that coumarins could be promising candidates against type-2 diabetes by restoring glomerular and islet structure through antioxidative mechanisms [55]. Some points need here with more clarifications. The physiology and molecular biology of the rodent pancreas are found to differ from those of humans. They have varied properties in their acinar cell physiology regarding exocytosis or signaling pathways. The ductal functions are also little different in human with comparison to the rodent pancreas [56]. Report suggests that mice are the most studied animal that resembles human pancreatic tissues. The histo-architecture of the endocrine islets and their composition has some differences. Arterial, venous, and lymphatic vessels innervations are also little different from species to species [57]. From biochemical point of views there are some differences; pyruvate carboxylase and ATP citrate lyase were significantly lower in human which suggests that human islets depend less on pyruvate carboxylation than rodents. On contrary, human islets possessed high levels of succinyl-CoA:3-ketoacid-CoA transferase, which are the regulator of acetoacetate formation. This suggests that human utilizes acetoacetate more than pyruvate carboxylase and citrate to form cytosolic acyl-CoA for calorie production [58].

So, from the current study in rat model and HepG2 cells results should be compared and extrapolated taking into account the structural/functional differences in these two species. Further studies are necessary in this regard.

## CONCLUSION

In summary, though the drugs used in the present investigation have opposite physiological functions (STZ as diabetes inducer and TLB as anti-diabetic), each exhibited some similar trend with ST expressions with the exception that TLB decreased PPST and DHAST in the human cells. Two drug/compounds have been examined in this study to discuss on drug-induced glycemic state-change. So, other drugs used for the same purpose have been discussed to extrapolate their possible role in SULTs expression and regulations of glycemic-state. Diabetogenesis is the result of complex interaction among altered hormones, metabolic and growth factors and their consequent signaling pattern. The specific roles of SULTs in the process of diabetogenesis need further investigation. Presently we are studying the effects of several other diabetogenic or anti-diabetic drugs on SULT expression and their related mechanisms in rat tissues and cultured human cells. This will help to evaluate the sulfation-metabolism of any molecule in the disease pathogenesis events.

## CURRENT AND FUTURE DEVELOPMENT

The current study elucidates the link between glucose homeostasis regulations and SULTs expressions. Glucose homeostasis has been modulated by some drugs and SULTs expressions and activities have been verified in human and rat tissues. The present study will be helpful for the understanding of the mechanism of diabetes in relation to the possible link of sulfation metabolism. New results from future investigation on SULTs expressions and possible sulfation metabolism of any biomolecules in diabetic condition may be exploited for therapeutic interventions.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study human and animal ethics guidelines were approved by the Institutional (Oriental Institute of Science and Technology, OIST/EC/Animal/ Human2018/012) Internal Review Board [IRB].

## HUMAN AND ANIMAL RIGHTS

All the human procedures were done in accordance with the Helsinki Declaration (2000) and National Institutes of Health (NIH) guidelines.

For all animal experiments, proper permissions were obtained from the Institutional (Oriental Institute of Science and Technology, OIST/EC/Animal/2018/012) Internal Review Board [IRB]. We followed all ethical norms and maintained requisite regulatory affairs.

## CONSENT FOR PUBLICATION

For human subjects, informed consent was obtained from all the participants of this study.

## AVAILABILITY OF DATA AND MATERIALS

The authors confirm that the data supporting the findings of this research is available within the article.

## FUNDING

None.

## CONFLICT OF INTEREST

The authors have no conflicts of interest, financial or otherwise.

## ACKNOWLEDGEMENTS

Declared none.

## REFERENCES

[1] Chapman, E.; Best, M.D.; Hanson, S.R.; Wong, C.H. Sulfotransferases: structure, mechanism, biological activity, inhibition, and synthetic utility. *Angew. Chem. Int. Ed. Engl.,* **2004**, *43*(27), 3526-3548. http://dx.doi.org/10.1002/anie.200300631 PMID: 15293241

[2] Crettol, S.; Petrovic, N.; Murray, M. Pharmacogenetics of phase I and phase II drug metabolism. *Curr. Pharm. Des.,* **2010**, *16*(2), 204-219. http://dx.doi.org/10.2174/138161210790112674 PMID: 19835560

[3] Hoda, M.; Hemaiswarya, S.; Doble, M. Pharmacokinetics and Pharmacodynamics of Polyphenols.*Role of Phenolic Phytochemicals in Diabetes Management*; Springer: Singapore, **2019**, pp. 159-173. http://dx.doi.org/10.1007/978-981-13-8997-9_7

[4] Potter, B.V.L. SULFATION PATHWAYS: Steroid sulphatase inhibition *via* aryl sulphamates: clinical progress, mechanism and future prospects. *J. Mol. Endocrinol.,* **2018**, *61*(2), T233-T252. http://dx.doi.org/10.1530/JME-18-0045 PMID: 29618488

[5] Mendis, E.; Kim, M.M.; Rajapakse, N.; Kim, S.K. Sulfated glucosamine inhibits oxidation of biomolecules in cells *via* a mechanism involving intracellular free radical scavenging. *Eur. J. Pharmacol.,* **2008**, *579*(1-3), 74-85. http://dx.doi.org/10.1016/j.ejphar.2007.10.027 PMID: 18036590

[6] Barbosa, A.C.S.; Feng, Y.; Yu, C.; Huang, M.; Xie, W. Estrogen sulfotransferase in the metabolism of estrogenic drugs and in the pathogenesis of diseases. *Expert Opin. Drug Metab. Toxicol.,* **2019**, *15*(4), 329-339. http://dx.doi.org/10.1080/17425255.2019.1588884 PMID: 30822161

[7] Foster, P.A.; Mueller, J.W. SULFATION PATHWAYS: Insights into steroid sulfation and desulfation pathways. *J. Mol. Endocrinol.,* **2018**, *61*(2), T271-T283. http://dx.doi.org/10.1530/JME-18-0086 PMID: 29764919

[8] Luo, L.; Zhou, C.; Hui, Y.; Kurogi, K.; Sakakibara, Y.; Suiko, M.; Liu, M.C. Human cytosolic sulfotransferase SULT1C4 mediates the sulfation of doxorubicin and epirubicin. *Drug Metab. Pharmacokinet.,* **2016**, *31*(2), 163-166. http://dx.doi.org/10.1016/j.dmpk.2016.01.003 PMID: 26948952

[9] Renskers, K.J.; Feor, K.D.; Roth, J.A. Sulfation of dopamine and other biogenic amines by human brain phenol sulfotransferase. *J. Neurochem.,* **1980**, *34*(6), 1362-1368. http://dx.doi.org/10.1111/j.1471-4159.1980.tb11216.x PMID: 6929898

[10] Alnouti, Y. Bile Acid sulfation: a pathway of bile acid elimination and detoxification. *Toxicol. Sci.,* **2009**, *108*(2), 225-246. http://dx.doi.org/10.1093/toxsci/kfn268 PMID: 19131563

[11] Maiti, S.; Chen, G. Methotrexate is a novel inducer of rat liver and intestinal sulfotransferases. *Arch. Biochem. Biophys.,* **2003**, *418*(2), 161-168. http://dx.doi.org/10.1016/j.abb.2003.08.019 PMID: 14522587

[12] Maiti, S.; Chen, G. Ethanol up-regulates phenol sulfotransferase (SULT1A1) and hydroxysteroid sulfotransferase (SULT2A1) in rat liver and intestine. *Arch. Physiol. Biochem.,* **2015**, *121*(2), 68-74. http://dx.doi.org/10.3109/13813455.2014.992440 PMID: 25720860

[13] Maiti, S.; Chen, X.; Chen, G. All-trans retinoic acid induction of sulfotransferases. *Basic Clin. Pharmacol. Toxicol.,* **2005**, *96*(1), 44-53. http://dx.doi.org/10.1111/j.1742-7843.2005.pto960107.x PMID: 15667595

[14] Jana, P.; Khan, M.M.; De, S.K.; Sinha, A.K.; Guha, S.; Khan, G.A.; Maiti, S. Estriol Inhibits Dermcidin Isoform-2 Induced Inflammatory Cytokine Expression *via* Nitric Oxide Synthesis in Human Neutrophil. *Curr. Mol. Med.,* **2018**, *18*(10), 672-678. http://dx.doi.org/10.2174/1566524019666190208095754 PMID: 30734678

[15] Zhai, T.; Wang, J.; Sun, L.; Chen, Y. The effect of streptozotocin and alloxan on the mRNA expression of rat hepatic transporters *in vivo*. *AAPS PharmSciTech,* **2015**, *16*(4), 767-770. http://dx.doi.org/10.1208/s12249-014-0262-0 PMID: 25549789

[16] Alimohammadi, S.; Hobbenaghi, R.; Javanbakht, J.; Kheradmand, D.; Mortezaee, R.; Tavakoli, M.; Khadivar, F.; Akbari, H. Protective and antidiabetic effects of extract from Nigella sativa on blood glucose concentrations against streptozotocin (STZ)-induced diabetic in rats: an experimental study with histopathological evaluation. *Diagn. Pathol.,* **2013**, *8*, 137. http://dx.doi.org/10.1186/1746-1596-8-137 PMID: 23947821

[17] Jensen, L.J.; Kuhn, M.; Stark, M.; Chaffron, S.; Creevey, C.; Muller, J.; Doerks, T.; Julien, P.; Roth, A.; Simonovic, M.; Bork, P.; von Mering, C.; Roth, A. STRING 8-a global view on proteins and their functional interactions in 630 organisms. *Nucleic Acids Res.,* **2009**, *37*, 412-416. http://dx.doi.org/10.1093/nar/gkn760

[18] Taboada, B.; Verde, C.; Merino, E. High accuracy operon prediction method based on STRING database scores. *Nucleic Acids Res.,* **2010**,

*38*(12), e130.
http://dx.doi.org/10.1093/nar/gkq254 PMID: 20385580

[19] von Mering, C.; Jensen, L.J.; Snel, B.; Hooper, S.D.; Krupp, M.; Foglierini, M.; Jouffre, N.; Huynen, M.A.; Bork, P. STRING: known and predicted protein-protein associations, integrated and transferred across organisms. *Nucleic Acids Res.,* **2005**, *33*(Database issue)(Suppl. 1), D433-D437.
http://dx.doi.org/10.1093/nar/gki005 PMID: 15608232

[20] Atlas, D. *International diabetes federation. IDF Diabetes Atlas,* 7th ed; International Diabetes Federation: Brussels, Belgium, **2015**.

[21] Chen, F.; Li, D.Y.; Zhang, B.; Sun, J.Y.; Sun, F.; Ji, X.; Qiu, J.C.; Parker, R.B.; Laizure, S.C.; Xu, J. Alterations of drug-metabolizing enzymes and transporters under diabetic conditions: what is the potential clinical significance? *Drug Metab. Rev.,* **2018**, *50*(3), 369-397.
http://dx.doi.org/10.1080/03602532.2018.1497645 PMID: 30221555

[22] Larson, E.C.; Pond, C.D.; Rai, P.P.; Matainaho, T.K.; Piskaut, P.; Franklin, M.R.; Barrows, L.R. Traditional preparations and methanol extracts of Medicinal plants from Papua New Guinea exhibit similar cytochrome p450 inhibition. *Evid. Based Complement. Alternat. Med.,* **2016**, *2016*
http://dx.doi.org/10.1155/2016/7869710 PMID: 27642356

[23] Gomes, A.I.P.V. *in vitro* assessment of cytochrome P450 2C9 inhibition: tolbutamide as probe substrate and HepaRG cells as human hepatic model (Doctoral dissertation). **2016**.

[24] Easterbrook, J.; Lu, C.; Sakai, Y.; Li, A.P. Effects of organic solvents on the activities of cytochrome P450 isoforms, UDP-dependent glucuronyl transferase, and phenol sulfotransferase in human hepatocytes. *Drug Metab. Dispos.,* **2001**, *29*(2), 141-144.
PMID: 11159803

[25] Bai, Q.; Zhang, X.; Xu, L.; Kakiyama, G.; Heuman, D.; Sanyal, A.; Pandak, W.M.; Yin, L.; Xie, W.; Ren, S. Oxysterol sulfation by cytosolic sulfotransferase suppresses liver X receptor/sterol regulatory element binding protein-1c signaling pathway and reduces serum and hepatic lipids in mouse models of nonalcoholic fatty liver disease. *Metabolism,* **2012**, *61*(6), 836-845.
http://dx.doi.org/10.1016/j.metabol.2011.11.014 PMID: 22225954

[26] Fan, L.Q.; You, L.; Brown-Borg, H.; Brown, S.; Edwards, R.J.; Corton, J.C. Regulation of phase I and phase II steroid metabolism enzymes by PPAR alpha activators. *Toxicology,* **2004**, *204*(2-3), 109-121.
http://dx.doi.org/10.1016/j.tox.2004.06.018 PMID: 15388238

[27] Li, Y.; Xu, Y.; Li, X.; Qin, Y.; Hu, R. Effects of PPAR-α agonist and IGF-1 on estrogen sulfotransferase in human vascular endothelial and smooth muscle cells. *Mol. Med. Rep.,* **2013**, *8*(1), 133-139.
http://dx.doi.org/10.3892/mmr.2013.1483 PMID: 23685729

[28] Gupte, A.A.; Pownall, H.J.; Hamilton, D.J. Estrogen: an emerging regulator of insulin action and mitochondrial function. *J. Diabetes Res.,* **2015**, *2015*
http://dx.doi.org/10.1155/2015/916585 PMID: 25883987

[29] Sharer, J.E.; Shipley, L.A.; Vandenbranden, M.R.; Binkley, S.N.; Wrighton, S.A. Comparisons of phase I and phase II *in vitro* hepatic enzyme activities of human, dog, rhesus monkey, and cynomolgus monkey. *Drug Metab. Dispos.,* **1995**, *23*(11), 1231-1241.
PMID: 8591724

[30] Prueksaritanont, T.; Gorham, L.M.; Hochman, J.H.; Tran, L.O.; Vyas, K.P. Comparative studies of drug-metabolizing enzymes in dog, monkey, and human small intestines, and in Caco-2 cells. *Drug Metab. Dispos.,* **1996**, *24*(6), 634-642.
PMID: 8781778

[31] Kirkpatrick, R. B.; Kraft, B. G. Effect of streptozotocin-induced diabetes on bile acid sulfation in male rat liver *American Journal ofa Physiology-Gastrointestinal and Liver Physiology,* **1984**, *247*(3), G226-G230.
http://dx.doi.org/10.1152/ajpgi.1984.247.3.G226

[32] Lv, L.; Wu, S.Y.; Wang, G.F.; Zhang, J.J.; Pang, J.X.; Liu, Z.Q.; Xu, W.; Wu, S.G.; Rao, J.J. Effect of astragaloside IV on hepatic glucose-regulating enzymes in diabetic mice induced by a high-fat diet and streptozotocin. *Phytother. Res.,* **2010**, *24*(2), 219-224.
http://dx.doi.org/10.1002/ptr.2915 PMID: 19610026

[33] Unger, E.; Pettersson, I.; Eriksson, U.J.; Lindahl, U.; Kjellén, L. Decreased activity of the heparan sulfate-modifying enzyme glucosaminyl N-deacetylase in hepatocytes from streptozotocin-diabetic rats. *J. Biol. Chem.,* **1991**, *266*(14), 8671-8674.
PMID: 2026583

[34] Almási, A.; Pinto, É.D.I.L.N.; Kovács, N.P.; Fischer, T.; Markovics, Z.; Fischer, E.; Perjési, P. Changes in hepatic metabolic enzyme activities and biliary excretion of 4-nitrophenol in streptozotocin induced diabetic rats. *Braz. J. Pharm. Sci.,* **2018**, *54*(1)
http://dx.doi.org/10.1590/s2175-97902018000117347

[35] Komatsu, K.; Ito, K.; Nakajima, Y.; Kanamitsu, Si.; Imaoka, S.; Funae, Y.; Green, C.E.; Tyson, C.A.; Shimada, N.; Sugiyama, Y. Prediction of *in vivo* drug-drug interactions between tolbutamide and various sulfonamides in humans based on *in vitro* experiments. *Drug Metab. Dispos.,* **2000**, *28*(4), 475-481.
PMID: 10725317

[36] Bhattacharya, S.; Ghosh, R.; Maiti, S.; Khan, G.A.; Sinha, A.K. The activation by glucose of liver membrane nitric oxide synthase in the synthesis and translocation of glucose transporter-4 in the production of insulin in the mice hepatocytes. *PLoS One,* **2013**, *8*(12)
http://dx.doi.org/10.1371/journal.pone.0081935 PMID: 24349154

[37] Jana, P.; Maiti, S.; Ghosh, R.; Ghosh, T.K.; Sinha, A.K. Estriol, a stimulator of nitric oxide synthesis in platelets, and its role as the powerful inhibitor of platelet aggregation. *Cardiovascular Endocrinology & Metabolism,* **2013**, *2*(3), 50-54.
http://dx.doi.org/10.1097/XCE.0b013e328362e40e

[38] Riazi, S.; Maric, C.; Ecelbarger, C.A. 17-beta Estradiol attenuates streptozotocin-induced diabetes and regulates the expression of renal sodium transporters. *Kidney Int.,* **2006**, *69*(3), 471-480.
http://dx.doi.org/10.1038/sj.ki.5000140 PMID: 16514430

[39] Fukui, M.; Kitagawa, Y.; Kamiuchi, K.; Hasegawa, G.; Yoshikawa, T.; Nakamura, N. Association between serum estradiol concentrations and carotid atherosclerosis in men with type 2 diabetes mellitus. *Metabolism,* **2008**, *57*(2), 285-289.
http://dx.doi.org/10.1016/j.metabol.2007.09.014 PMID: 18191062

[40] Li, Y.; Huang, J.; Yan, Y Preventative effects of resveratrol and estradiol on streptozotocin-induced diabetes in ovariectomized mice and the related mechanisms *PLoS One,* **2018**, *13*(10), e0204499.
http://dx.doi.org/10.1371/journal.pone.0204499

[41] Xu, Y.; Yang, X.; Wang, Z.; Li, M.; Ning, Y.; Chen, S.; Yin, L.; Li, X. Estrogen sulfotransferase (SULT1E1) regulates inflammatory response and lipid metabolism of human endothelial cells *via* PPARγ. *Mol. Cell. Endocrinol.,* **2013**, *369*(1-2), 140-149.
http://dx.doi.org/10.1016/j.mce.2013.01.020 PMID: 23384540

[42] Yalcin, E.B.; Kulkarni, S.R.; Slitt, A.L.; King, R. Bisphenol A sulfonation is impaired in metabolic and liver disease. *Toxicol. Appl. Pharmacol.,* **2016**, *292*, 75-84.
http://dx.doi.org/10.1016/j.taap.2015.12.009 PMID: 26712468

[43] Garbacz, W.G.; Jiang, M.; Xie, W. Sex-Dependent Role of Estrogen Sulfotransferase and Steroid Sulfatase in Metabolic Homeostasis. *Adv. Exp. Med. Biol.,* **2017**, *1043*, 455-469.
http://dx.doi.org/10.1007/978-3-319-70178-3_21 PMID: 29224107

[44] Li, J.; Wei, J.; Xu, P.; Yan, M.; Li, J.; Chen, Z.; Jin, T. Impact of diabetes-related gene polymorphisms on the clinical characteristics of type 2 diabetes Chinese Han population. *Oncotarget,* **2016**, *7*(51), 85464-85471.
http://dx.doi.org/10.18632/oncotarget.13399 PMID: 27863428

[45] Zhou, X.; Rougée, L.R.; Bedwell, D.W.; Cramer, J.W.; Mohutsky, M.A.; Calvert, N.A.; Moulton, R.D.; Cassidy, K.C.; Yumibe, N.P.; Adams, L.A.; Ruterbories, K.J. Difference in the Pharmacokinetics and Hepatic Metabolism of Antidiabetic Drugs in Zucker Diabetic Fatty and Sprague-Dawley Rats. *Drug Metab. Dispos.,* **2016**, *44*(8), 1184-1192.
http://dx.doi.org/10.1124/dmd.116.070623 PMID: 27217490

[46] Runge-Morris, M.; Vento, C. Effects of streptozotocin-induced diabetes on rat liver sulfotransferase gene expression. *Drug Metab. Dispos.,* **1995**, *23*(4), 455-459.
PMID: 7600911

[47] Wang, X.; Wang, F.; Zhang, Y.; Xiong, H.; Zhang, Y.; Zhuang, P.; Zhang, Y. Diabetic cognitive dysfunction is associated with increased bile acids in liver and activation of bile acid signaling in intestine. *Toxicol. Lett.,* **2018**, *287*, 10-22.
http://dx.doi.org/10.1016/j.toxlet.2018.01.006 PMID: 29382564

[48] Meng, X.M.; Ma, X.X.; Tian, Y.L.; Jiang, Q.; Wang, L.L.; Shi, R.; Ding, L.; Pang, S.G. Metformin improves the glucose and lipid metabolism *via* influencing the level of serum total bile acids in rats with streptozotocin-induced type 2 diabetes mellitus. *Eur. Rev. Med. Pharmacol. Sci.,* **2017**, *21*(9), 2232-2237.
PMID: 28537659

[49] Chen, Z.; Hu, H.; Chen, M.; Luo, X.; Yao, W.; Liang, Q.; Yang, F.; Wang, X. Association of Triglyceride to high-density lipoprotein cholesterol ratio and incident of diabetes mellitus: a secondary retrospective analysis based on a Chinese cohort study. *Lipids Health Dis.,* **2020**, *19*(1), 33.
http://dx.doi.org/10.1186/s12944-020-01213-x PMID: 32131838

[50] Bi, Y.; Wang, Y.; Xie, W. The interplay between hepatocyte nuclear factor 4α (HNF4α) and cholesterol sulfotransferase (SULT2B1b) in hepatic energy homeostasis. *Liver Research,* **2019**, *3*(3-4), 143-149.
http://dx.doi.org/10.1016/j.livres.2019.09.004

[51] Garbacz, W.G.; Jiang, M.; Xu, M.; Yamauchi, J.; Dong, H.H.; Xie, W. Sex- and Tissue-Specific Role of Estrogen Sulfotransferase in Energy Homeostasis and Insulin Sensitivity. *Endocrinology,* **2017**, *158*(11), 4093-4104.
http://dx.doi.org/10.1210/en.2017-00571 PMID: 28938414

[52] Dong, B.; Saha, P.K.; Huang, W.; Chen, W.; Abu-Elheiga, L.A.; Wakil, S.J.; Stevens, R.D.; Ilkayeva, O.; Newgard, C.B.; Chan, L.; Moore, D.D. Activation of nuclear receptor CAR ameliorates diabetes and fatty liver disease. *Proc. Natl. Acad. Sci. USA,* **2009**, *106*(44), 18831-18836.
http://dx.doi.org/10.1073/pnas.0909731106 PMID: 19850873

[53] Chen, X.; Maiti, S.; Zhang, J.; Chen, G. Nuclear receptor interactions in methotrexate induction of human dehydroepiandrosterone sulfotransferase (hSULT2A1). *J. Biochem. Mol. Toxicol.,* **2006**, *20*(6), 309-317.
http://dx.doi.org/10.1002/jbt.20149 PMID: 17163485

[54] Chen, X.; Zhang, J.; Baker, S.M.; Chen, G. Human constitutive androstane receptor mediated methotrexate induction of human dehydroepiandrosterone sulfotransferase (hSULT2A1). *Toxicology,* **2007**, *231*(2-3), 224-233.
http://dx.doi.org/10.1016/j.tox.2006.12.019 PMID: 17276571

[55] Yao, Y.; Zhao, X.; Xin, J.; Wu, Y.; Li, H. Coumarins improved type 2 dia-

betes induced by high-fat diet and streptozotocin in mice *via* antioxidation. *Can. J. Physiol. Pharmacol.,* **2018**, *96*(8), 765-771. http://dx.doi.org/10.1139/cjpp-2017-0612 PMID: 29641229

[56] Case, R.M. Is the rat pancreas an appropriate model of the human pancreas? *Pancreatology,* **2006**, *6*(3), 180-190. http://dx.doi.org/10.1159/000091849 PMID: 16534243

[57] Dolenšek, J.; Rupnik, M.S.; Stožer, A. Structural similarities and differences between the human and the mouse pancreas. *Islets,* **2015**, *7*(1) http://dx.doi.org/10.1080/19382014.2015.1024405 PMID: 26030186

[58] MacDonald, M.J.; Longacre, M.J.; Stoker, S.W.; Kendrick, M.; Thonpho, A.; Brown, L.J.; Hasan, N.M.; Jitrapakdee, S.; Fukao, T.; Hanson, M.S.; Fernandez, L.A.; Odorico, J. Differences between human and rodent pancreatic islets: low pyruvate carboxylase, atp citrate lyase, and pyruvate carboxylation and high glucose-stimulated acetoacetate in human pancreatic islets. *J. Biol. Chem.,* **2011**, *286*(21), 18383-18396. http://dx.doi.org/10.1074/jbc.M111.241182 PMID: 21454710