Research Square

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Modulation of Ras GTPase and IRS-1 Activities by Spermine in Type 2 Diabetes: A Study Using Goto Kakizaki Rat Model

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Abstract

In this study we investigated the biochemical relationship between spermine, Ras GTPase activity, and insulin signaling in Goto Kakizaki (GK) rats, a model for type 2 diabetes mellitus. Ras GTPases regulate cellular signaling by cycling between active GTP-bound and inactive GDP-bound states, controlled by Guanine Nucleotide Exchange Factors (GEFs) and GTPase-Activating Proteins (GAPs). Our aim is to determine whether spermine modulates this pathway to improve insulin responsiveness.

Over six weeks, varying doses of spermine were administered, and Ras GTPase activity, Total Insulin Receptor Substrate-1 (IRS-1) levels, and blood glucose concentrations were monitored with a luminometer. Increased luminometer readings (80,000–120,000 RLU) indicated enhanced Ras GTPase activity, associated with elevated GEF activity and reduced GAP activity. This upregulation corresponded with increased IRS-1 concentrations, suggesting corresponding improvement in insulin signaling.

From our findings, spermine demonstrated a dose-dependent hypoglycemic effect. With higher doses (25 mg/mL and 12.5 mg/mL) significantly reduced blood glucose levels compared to lower doses. Notably, 25 mg/mL spermine reduced glucose levels from 12.40 mmol/L to 9.80 mmol/L, representing a 21.6% decrease.

These findings reveal that spermine enhances Ras GTPase activity and IRS-1 expression, leading to improved glucose regulation, suggesting a potential biochemical pathway through which spermine may restore insulin sensitivity. The study highlights Ras GTPase modulation as a promising therapeutic target for improving cellular responsiveness to insulin in type 2 diabetes management.

1. Introduction

The mammalian pancreas is composed of several cells which play vital roles in energy metabolism. These cells co-exist together in a physiologically homeostatic enclosure, while eliciting their functions in paracrine or endocrine mode [1]. The islet cells of the pancreas are made up of five (5) different cells, existing in varied percentages and responsible for different functions: (1) Alpha-cells secrete glucagon, which raises blood glucose levels; (2) Beta-cells produce insulin, which regulates blood sugar levels; (3) Delta cells secrete somatostatin, which inhibits the release of insulin and glucagon and (4) Epsilon cells produce pancreatic polypeptide, which regulates pancreatic exocrine function and glucose metabolism [2]. Despite their distinct functions, these cells work together in a complex regulatory network to maintain energy homeostasis [2, 20]. The unique biochemical and cellular interplay in pancreas cell organization and distribution of most mammalian species are constantly been studied, to gain more insights for similarity between human and experimental animals as scientist attempts to study and propose effective management of diabetes mellitus [3].

Presently, diabetes mellitus is still remain a life-threatening disease, with varied complications ranging from retinopathy, nephropathy, neuropathy, infertility and cardiovascular diseases [19]. Overall, diabetes mellitus is classified into two types including type I diabetes mellitus and type 2 diabetes mellitus. Both,

differ significantly. In type 1 diabetes mellitus, β cells of pancreas are disrupted and it is considered as an autoimmune disease, leading to impaired insulin secretion [14]. However, the insulin levels are relatively high in type 2 diabetes mellitus but cells are resistant to the insulin, which is present [4].

In the work of [7], they reported in their work, validating the alternating regulatory switch of GTPase in coordinating cell membrane traffic, and as the central biochemical process for determining cell survival and cell death as described by [16]. Similarly, most recently [9], provided insights into Ras GTPase effector protein, with a significant background of how Ras-GTPase mediate signaling process, which has great therapeutic potential, revealing Ras GTPase as central for future research into human disease, phenotype therefrom, due to its ability to promote protein-protein interaction, and cell signal activations [8]. This further corroborated [9], which had established the biochemical connection between Ras GTPase and the activation of Mitogen Activated Protein Kinase, a major protein-kinase that drive and promotes cell signaling, cell to cell interaction, and cellular regeneration processes. Thus, research into bioactive compounds or secondary metabolites that could possibly promote Ras GTPase activity in diabetic GK rats, will be essential as it may provide the needed prism to peers into what may happens to Ras GTPase activity in diabetic and non-diabetic patients. Moreover, most receptor proteins and signal activators are membrane bound, the activation and deactivation function of the GTPases and their receptors has become the determining factor for cell sensitivity to protein-protein interactions and cell to cell signaling for a wide range of biomolecules entering and exiting through the cell membrane [21, 28].

IRS-1 serves as the receptor link or docking proteins; whose biochemical state either favour or disfavour insulin binding, the later, being the physiological situation in type 2 diabetes. In the works of [32], they reported how activation of IRS-1 could be linked to type 2 diabetes, revealing that biochemical link exists between the signaling pathway of IRS-1 activation through phosphorylation reaction codes.

The Goto Kakizaki (GK) Rat is a suitable animal model for experimenting on type 2 diabetes mellitus. This strain of rat has been used extensively by scientist including; [12, 23], to study type 2 diabetes and also, to explore the genetic and physiological characterization of this disease phenotype.

The GK rat's characteristics makes it the most preferred animal models for studying non-obese type 2 diabetes [23]. GK rat was first developed by selective in-breeding of wistar rats, after successive in-breeding process, to get a new strain of GK rat with genetic impairment for glucose tolerance, which is characterized by hyperglycemia, nephropathy and retinopathy from 8–10 weeks of age [11, 12]. GK rat polygenic strain characterization has been studied and these was found to reside on three independent loci in chromosome 7, which is involved in type 2 diabetes [23].

Spermine is a bioactive compound of very important physiological relevance which promotes cell to cell signaling, cell regeneration and cell growth in eukaryotic cells. [20], described the potential role of leveraging the healthy cell regenerative ability, influenced by permine in ancer and malignancy. Spermine and other polyamines have been reported as essential biomolecules responsible for normal cellular metabolism in humans [6]. In the overview study of polyamines by [31], he revealed the tumor and cancer suppressing effects of spermine and spermidine, through DNA methylation of targeted pathological

cells, corroborating that polyamines have become bioactive compounds of therapeutic interest in life science research.

Amongst other polyamines, spermine is considered as a very important polyamine produced by mammalian cells, that is involved in ion channels regulation and inhibition of lipid formation [20]. Recent studies show that spermine holds the potential of promoting immune responses and also triggering cell to cell communication in mediating cellular metabolites homeostasis [16, 20]. Several biochemical studies in recent decade seeks to unravel the underlining biochemical connections between polyamine and diseases of metabolic disorders [20]. Polyamines have been reported to play a vital role in the growth and differentiation of the β cells of the pancreas, where they have been associated with proinsulin biosynthesis and secretion of insulin [22]. They are present in millimolar (mM) concentrations in both eukaryotic and Prokaryotic cells [17, 22]. Moreover, different genes in eukaryotes and their components are modulation driven by polyamines bearing, polyamine encoded proteins are involved in transcriptional processes [14, 17].

In this work, our focus is to understand the biochemical role of spermine on GTPase and IRS-1 activities, and how this can affects cellular response to insulin, in type 2 diabetes.

2 Materials and Methods

Chemicals and Reagents

All materials used for this study were of analytical and molecular grade and purchased from the following companies: GTPase reagent, GloMax Luminescence assay kits (Promega Corperation, USA), Glucose meter (Accu- Check), Spermine (MedChemExpress, USA), Dimethylsulfoxide 96% DMSO (MedChem Express, USA), Ethanol (Sigma-Aldrich, USA) Luminometer (version V301001 (VITL Tech, Germany) and Goto Kakizaki (GK) rats were purchased from (CLEA, Japan)

Methods

Experimental animals

Materials

Goto Kakizaki (GK) rats, purchased from CLEA, Japan, were housed in animal facility at room temperature with a 12 hours day/night cycle and given unlimited access to standard rat feed (Top feeds, Sapele, Delta State, Nigeria) and water ad libitum, at the Animal house. The procedure for animal handling and experimentation was approved by Institutional Ethical Committee, on animal use, University of Abuja, with ethical clearance number (**UAECAU/2024/018**) in line with the Helsinki Declaration of [25], as revised in [26], and also the National Institute of Health (NIH) Guidelines for the care and use of Laboratory Animal [30].

Clinical Trial Number: not applicable.

Confirmation Test

Upon arrival of the GK rats, two (2) biochemical confirmatory assays and one physical observation test were carried out. To confirm nephropathy, Blood Urea Nitrogen (BUN) serum assay was conducted following manufacture's protocol (Roche Cobas 6000(c501 module) BUN assay kit. Hyperglycemia was determined following the method of [33]. To confirm retinopathy, in the absence of non-mydriatic camera, physical examination for dilated intraretinal hemorrhage and cotton-wool spot.

Animal Grouping

Fifteen (15) male Goto Kakizaki (GK) rats were assigned to five Groups of three (3) rats per group (n = 3). Animals in Group A were administered (25.00mg/mL), Groups B (12.50mg/mL), Group C (6.25mg/mL), Group D (3.13mg/mL) of spermine and Group E (Control group). An Accu-Chek glucometer was used to determine the blood glucose concentrations of all the experimental animals [13]. Male GK rats were used for the experiment due to known metabolic and hormonal differences between the sexes. Also we used male rats for this study to avoid the influence of female sex hormones on some of the parameters we are evaluating.

Methods

The preparation of all reagents was done according to manufacturer's instructions in a well illuminated Laminar flow hood. The preparations were appropriately labeled as follows:

Reagent A

Exactly 15µL aliquot of GTPase-Glo-reagent which is pre-diluted and optimized for Ras GTPase, was transferred into the GTPase-Glo-buffer. Also, 50µL of 10mM ADP was transferred into a 5 mL GTPase-Glo-buffer and labelled A. Reagent A is now the reconstituted GTPase-Glo-reagent.

Reagent B

Four 5mL sterile screw cap reagent bottles were labelled 3.13mg/mL, 6.25mg/mL, 12.50mg/mL and 25.00mg/ml. Exactly 2mL DMSO was pipetted into the four sterile screw cap reagent bottles. To the reagent bottle labelled 25mg/mL, 3.13mg/ml of spermine was weighed, using a digital weighing balance (Mettler) and subsequently transferred into a 2 mL DMSO in the reagent bottle. The constituent in the reagent bottle was thoroughly mixed and kept at room temperature. To the reagent bottle labelled 6.25mg/mL, 6.25mg/mL spermine was weighed into the sample bottle and mixed. The same process was repeated for 12.50mg/mL and 25.00mg/mL. All reagents were properly mixed before administrating the same to the GK rats.

LD50 Determination for Spermine

An initial acute toxicity (LD_{50}) test was carried out according to [29] with minor modifications to determine nonlethal dose concentrations of 25mg/mL, 12.5mg/mL, 6.25mg/mL and 3.13mg/mL of

spermine, which were used as the concentrations of spermine, found tolerable to the GK rats.

Evaluation of the Effect of Spermine on Ras GTPase activity

The evaluation of the effects of Spermine on GTPase activity was done following established protocol as contained in Promega GTPase luminescence protocol (V7681 and V7682, 2020). 0.5ml blood was collected from each animal into a 2mL EDTA test tube, centrifuged at 7000rpm for 10 minutes, to extract the serum. Pre-optimized Ras GTPase buffer was aliquot into the sample test tube containing 2µL serum, 5µL of the 2X GTP reaction solution was added and incubated at 25°C for 1 hour. 5µL detection reagent was added and luminescence read with the luminometer

The administration of Spermine, was carried out once a week for each group for 6 weeks across all groups at different concentrations of 3.13mg/mL, 6.25mg/mL, 12.50 mg/mL and 25.00mg/mL. All the assays were done before the commencement of administration of polyamines, and also at the end of week 1, 3 and 6 across all groups.

Total IRS-1 concentrations were measured using the Elabscience IRS-1 ELISA kit (Cat. Number: E-EL-H554) according to the manufacturer's protocol. 2mL blood was collected from animals in all groups into a 5mL test tube, centrifuge at 7000RPM for 10 minutes. 2uL serum was aliquot into 96well pre-coated IRS-1 antibody microplate tubes.10uL biotinylated detection reagent was added to each well, sealed and incubated for 1 hour at 37℃10uL Avidin-HRP conjugate was added and also incubated for 1 hour at 37℃,and 5uL substrate reagent was added and finally incubated for 15minutes at 37℃.Absorbance 450nm.

Analysis of Results:

The data obtained from the study were analyzed using One Way ANOVA using Statistical Package for Social Sciences (SPSS) version 27. Results are presented as Mean ± SD. Level of significance was set at $P < 0.05$

3. Results

Figure 1. Show the baseline results of the effect of spermine on GTPase, measured in Relative Light Unit (RLU), before the commencement of administration of spermine.

Fig. 1: Baseline activities of Ras GTPase, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs) in GK rats prior to spermine administration. Data are presented as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was defined at $p < 0.05$. Values with identical superscript letters are not significantly different

Group A ▬ Before commencement of spermine administration

Group B ▬ Before commencement of spermine administration

Group C ▬ Before commencement of spermine administration

Group D ▬ Before commencement of spermine administration

Group E ▬ Control

The Figure 1: shows the mean values for GTPase GAPs and GEF activities, measured in Relative Light Unit (RLU) for GK rats across all groups, before the commencement of administration of spermine. Inactive Ras GTPase readings of 50000 (RLU), suggests that animals, yet to be subjected to spermine administration, showed no Ras GTPase, GAPs and GEF activities.

Fig. 2: Ras GTPase activity, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factor (GEF) activities in GK rats following one week of spermine administration. Data are expressed as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was set at $p < 0.05$. *Spm* denotes spermine. Values sharing identical superscript letters are not significantly different

Figure 2. untreated group showed varied luminometer readings. Active Ras GTPase, activities was recorded for group A and B, treated with 25.00mg/mL and 12.50mg/mL of spermine, and recording luminometer values of about 80000 ($8x10^{4)}$ (RLU), with corresponding increase in GEF activities for both groups. The results indicate gradual sensitivity of GTPase to spermine across treated groups, after the first week of spermine administration, GTPase remained inactive for groups C, D and the control group.

Fig. 3: Ras GTPase activity, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factor (GEF) activities in GK rats after three weeks of spermine administration. Data are presented as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was set at $p < 0.05$. *Spm* denotes spermine. Values sharing identical superscript letters are not significantly different.

Figure 4. Show the results for the effect of spermine on Ras GTPase, GAPs and GEF activities, at the end of the sixth week of spermine administration

Fig. 4: Illustrate the activities of Ras GTPase, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs) in GK rats following six weeks of spermine (Spm) administration. Data are expressed as mean ± standard error of the mean (SEM), with statistical significance set at $p < 0.05$. Superscripts sharing identical letters indicate no statistically significant difference between groups.

The results presented in Fig. 4 is the significant upregulation of Ras GTPase and GEF activities, which were observed across the treated groups, accompanied by a corresponding downregulation of GAP activity. Notably, these changes were already evident by week 3 of spermine administration and persisted through week 6.

Conversely, an increase in GTPase activities translated to a decrease in GAPs activities. The control group however, remained inactive from week one (1) through the six (6) weeks of spermine treatment at varied concentrations.

Below is Figure 5, which shows the results for the effect of spermine on IRS-1 Concentration, across different groups of varied concentration, before commencement (BC), and at the end of weeks 1, 3 and 6 of spermine administration.

Fig. 5: depicts total insulin receptor substrate-1 (IRS-1) concentrations in GK rats prior to spermine administration and at the end of weeks 1, 3, and 6 of treatment. Baseline values, denoted as (BC), represent measurements obtained before the initiation of spermine exposure.

Figure 5. Shows the effect of spermine administration on total IRS-1 concentration in GK rats before commencement of spermine administration. The total IRS-1 concentration was markedly reduced at baseline (0.68 mg/mL) but increased significantly following spermine administration, reaching 5.46 mg/mL in GK rats treated with 25.00 mg/mL spermine by week 6 of the experimental period.

Notably, results of week 6, recorded the highest total IRS-1 concentration at 25mg/ml spermine treatment.

The Figure 6 below Shows the results for the effect of spermine on Blood Glucose Concentrations in GK Rats

Fig. 6: Illustrates blood glucose concentrations in GK rats prior to the initiation of spermine administration and at the end of weeks 1, 3, and 6 of treatment. Baseline measurements, denoted as (BC), represent values obtained before spermine exposure

Figure 6. Shows the effect of varied concentrations of spermine on blood glucose concentrations in GK rats. There was a significant decrease in glucose level from 12.43mmol/L to 9.80 mmol/L for group A, which were treated with 25.00mg/mL spermine after 6 weeks of spermine administration, making it the best performing decreased glucose concentration for the effect of spermine on blood glucose concentration in GK rats. Glucose levels also dropped significantly for other treated groups B, C and D, as compared to the control group E.

Table 1

Percentage (%) glucose decrease at the highest dose of spermine (25.0mg/mL)

Weeks	Mean blood glucose Conc. (mmol/L)	SD	Percentage (%) glucose Conc.
1	12.40	±1.7	0.80
3	10.80	±0.9	13.60
6	9.80	±1.2	21.60

Table 1, shows percentage decrease in glucose concentration following administration of high dose of spermine (25mg/ml) across treatment groups for the first, third and sixth week respectively. Glucose

concentration dropped from 12.40mmol/L at the end of the first week of spermine administration to 9.80mmol/L at the end of the sixth week of spermine administration, representing a 21.60% decrease.

4. Discussion

Results from this study show a biochemical link between spermine, Ras GTPase activities, total IRS-1 concentrations and glucose metabolism in GK rats. This is seen in the significant decrease in blood glucose concentrations after six (6) weeks of spermine administration.

By increasing Ras GTPase activities in dose dependent manner within after six weeks spermine treatment, animals in the treated groups recorded corresponding sharp increase in total IRS-1 concentrations, with significant decrease in blood glucose levels across all treated groups, while animals in the control group recorded no significant change in these biochemical parameters evaluated.

This research findings, agrees with the work of [32], who suggested a possible link between IRS-1 and the phosphorylation triggers of signaling proteins (PI3K).

It is known that Ras GTPase is active when it binds GTP, leading to concomitant signals transduction between cells, this signaling produces by Ras GTPase, is believed to be amplified through the phosphorylation cascade, which activates Phosphoinositol-3-kinase (PI3K). The activation of PI3K further facilitate the activation of IRS-1, and the activation IRS-1 further trigger the activation of facilitated glucose transport like the Glucose Transporter 4 (GLUT 4), as reported by [34]

Two (2) families of proteins – Guanine Activating Proteins (GAPs) and Guanine Nucleotide Exchange Factors (GEFs), are responsible for the switching process between the active and inactive state, bearing they mediates the alternating states of the GTPase [21]. GEFs binds the inactive GDP-GTPase, to replace GDP with GTP, enabling the switching on of the active GTP-GTPase state. GAPs are required to hydrolyze the GTP-GTPase bond complex, to release GTP from GTPase, and the remaining GTP, converted to GDP, ATP and inorganic phosphate (pi) [18, 28]. The amount of ATP released is measured as luminescence, by the Luminometer using the Promega GTPase-Glo assay kit wwhich is pre-optimized for quantitative Ras GTPase activity measurement [28].

The ATP measured by the luminometer is based on “luciferine-luciferase” working principle of the optimized Ras GTPase Promega reagent, which converts the available GTP after the GTPase-GTP reaction into GDP and ATP. Active GTPase results indicates an increased GEF activity of Ras GTPase bound to GTP, making GTP less available during the reaction process. Low GTP means less ATP detectable in the GTPase-Glo assay, signifying an active Ras GTPase-GTP state, hence, a sustained cell signaling process. Active Ras GTPase activity promotes signal transduction. However, inactive Ras GTPase activity signify low/no signal transduction. High GTP during the during the GTPase-Glo assay reaction means low activity of Ras GTPase, signal transduction is impaired at this point.

The results of varied concentrations of spermine across test groups and control group were recorded in triplicate and the mean value data obtained from this research were analyzed using SPSS Version 27. Statistical significance difference was set at $p < 0.05$.

Interestingly, GTPase activity progressively increased from the first week of commencement of administration to the sixth week, with proportional decrease in blood glucose values in treated groups. Across the groups, group 1 which was administered 25mg/mL spermine, recorded most optimal GTPase activities, with proportional decrease in blood glucose levels, dropping from 12.40mmol/L at the first week of administration to 9.80mmol/L, representing a 21.60% decrease at the end of week six of spermine administration.

Same increase in GTPase activities with proportional decrease in blood glucose level was recorded among animals of group that were administered 12.50mg/mL, 6.25mg/mL and 3.13mg/mL, with corresponding drop of glucose values from

This findings supports available biochemical reports that glucose Transporter Type 4 (GLUT 4) is insulin dependent, functioning to enhance glucose uptake in muscle and adipose tissue via the insulin Receptor Substrate 1 (IRS-1), whose activation, triggers the activation of GLUT 4, from a cascade of biochemical events, which we can now link to Ras GTPase signal activation as seen from the data presented in this study. It is established that, when GTPase is active, the enzyme Adenylate cyclase is activated, with a view to promote the conversion ATP to cAMP. The second messenger cAMP, facilitates the activation of the Protein Kinases including; protein kinase A, protein kinase B, phosphoinosol-3 kinase and macrophage activating protein kinases (PKA, PKB, P13K and MAPK) through phosphorylation reactions [17,18, 19]. It is through the sequence of these signaling and activation mechanism/processes that ultimately triggers Insulin Receptor Substrate – 1, which promotes the GLUT 4 glucose trafficking from extracellular space, enhancing glucose utilization and maintaining homeostasis [18,19].

The results as presented in this research, further enhances available scientific data, that, bioactive compound like spermine are important in enhancing insulin sensitivity bearing, it promotes glucose utilization in diabetic GK rats, a non-obese model, known to be genetically stable with consistent phenotype. Such are possible through the involvement of spermine in further promoting the up-regulation of cell signaling activities, through phosphorylation process involving the kinases [17,19].

Conclusion

The results from this research shows, direct up-regulation of Ras GTPase activity in diabetic GK rats by the polyamine - specifically spermine. This up-regulated GTPase activity was observed to proportionately account for a sharp decrease in blood glucose levels in diabetic GK rats. Spermine concentration of 25.00mg/mL decreased glucose level from 12.40mmol/L to 9.80mmol/L in GK rats after six weeks of administration, representing a 21.60% decrease in blood glucose concentration, suggesting that spermine's effects on glucose levels is concentration dependent. Specifically, concentrations of 25.00mg/mL and 12.50mg/mL of spermine were found to be more effective in reducing blood glucose

levels in diabetic GK rats, than concentrations of 6.25mg/ml and 3.13mg/ml. Hence, 25.00mg/mL of Spermine supplementation may be more beneficial in reducing hyperglycemia and ultimately in management of type 2 Diabetes Mellitus. Undoubtedly, further studies are needed to have a full understanding of the molecular mechanisms underlying spermine's effects on Ras GTPase activity and cellular signaling, warranting clinical trials to evaluate the safety and efficacy of spermine as a therapeutic agent for type 2 DM.

Declarations

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Author Contribution

B.O.O. Conceptualization, Methodology, Original draft preparation. LA.A: Conceptualization, Data curation, original drafting preparation, Visualization. A.I.O. Methodology, Data validation, original draft preparation. K.C.N. Draft preparation, Data validation, visualization. B.A.I Data validation, Methodology, curation. M.P.O. Conceptualization, Supervision, Data Investigation, Writing reviewing and editing.

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Figures

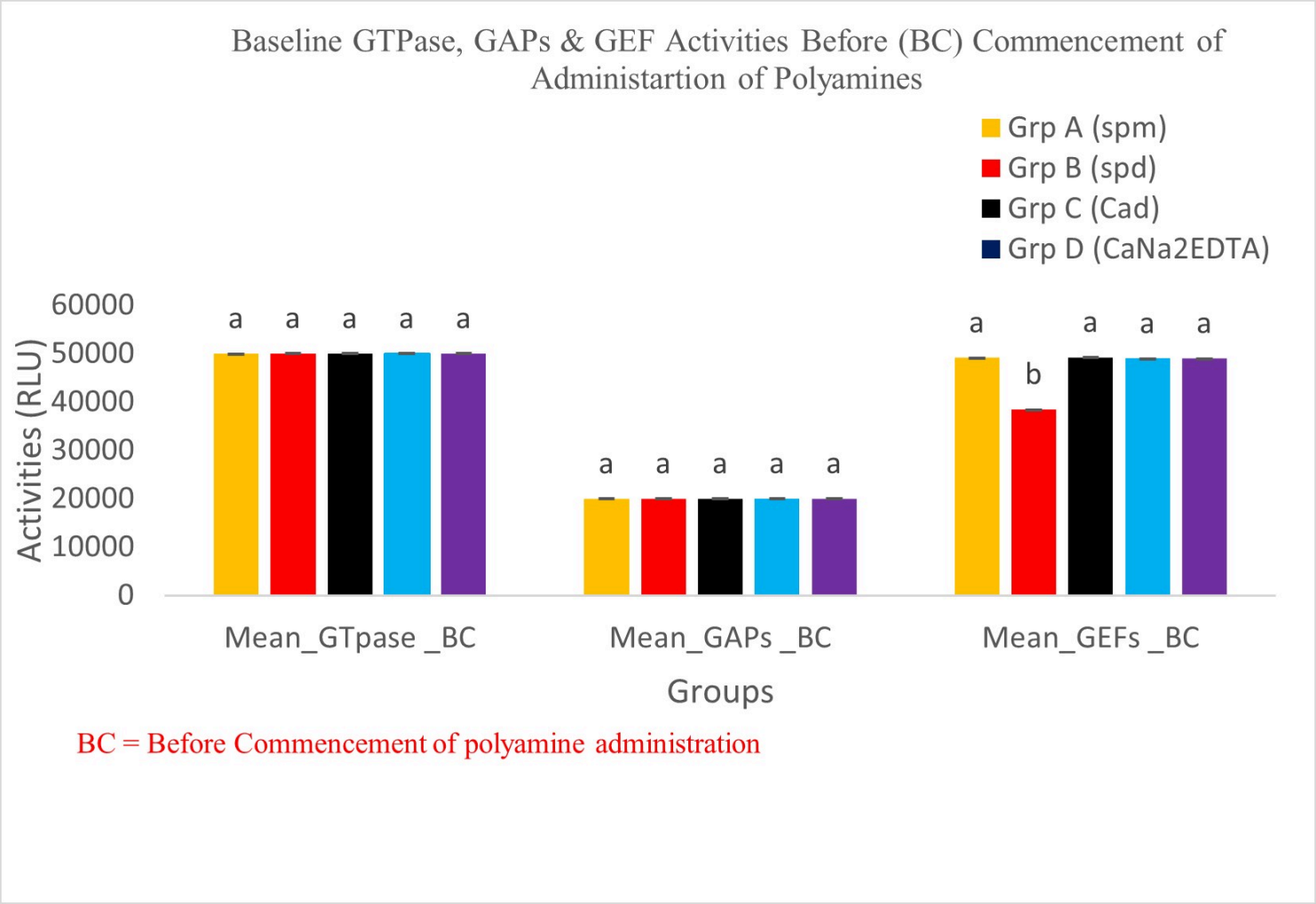


Figure 1

Baseline activities of Ras GTPase, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs) in GK rats prior to spermine administration. Data are presented as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was defined at $p < 0.05$. Values with identical superscript letters are not significantly different

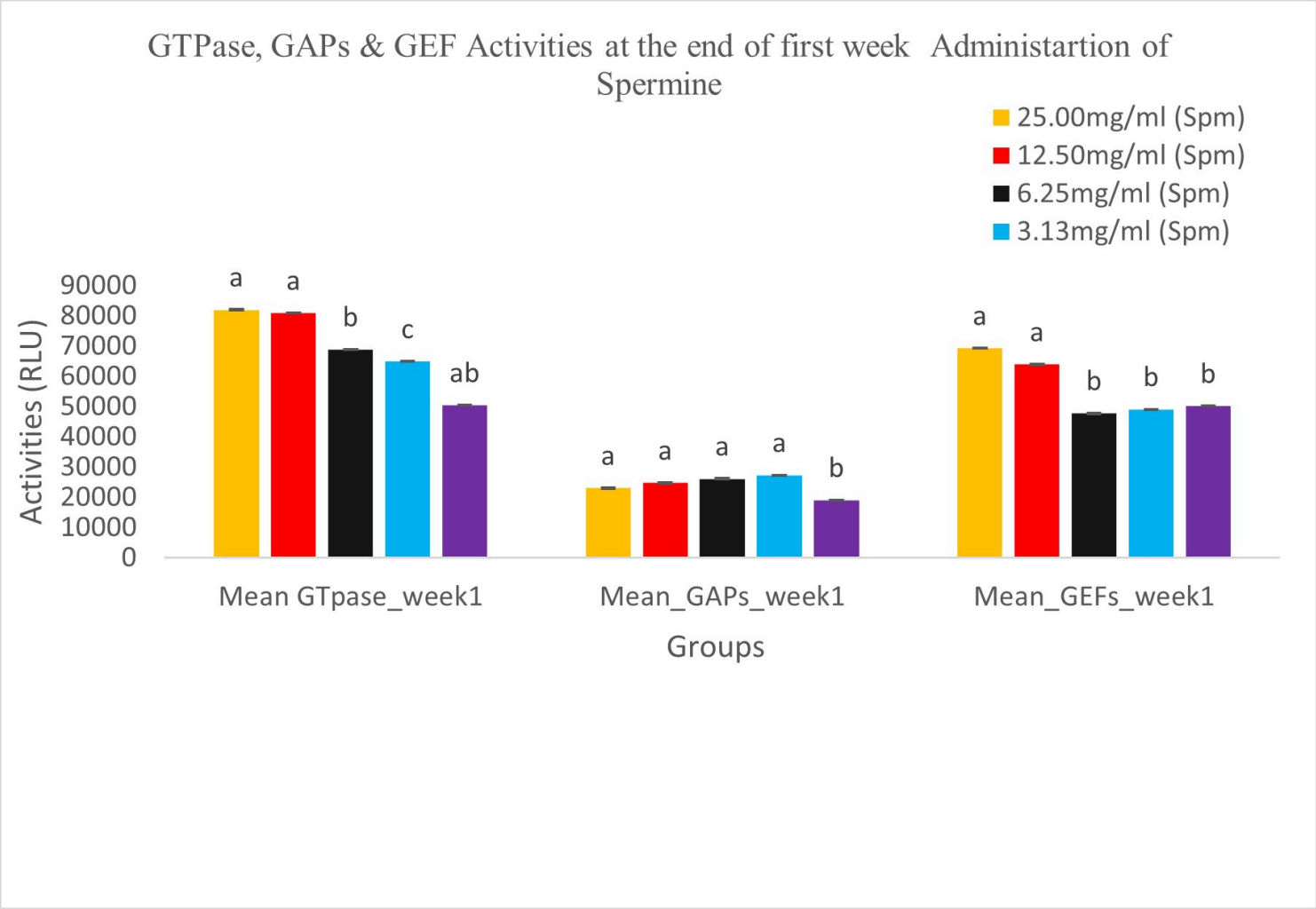


Figure 2

Ras GTPase activity, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factor (GEF) activities in GK rats following one week of spermine administration. Data are expressed as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was set at $p < 0.05$. *Spm* denotes spermine. Values sharing identical superscript letters are not significantly different

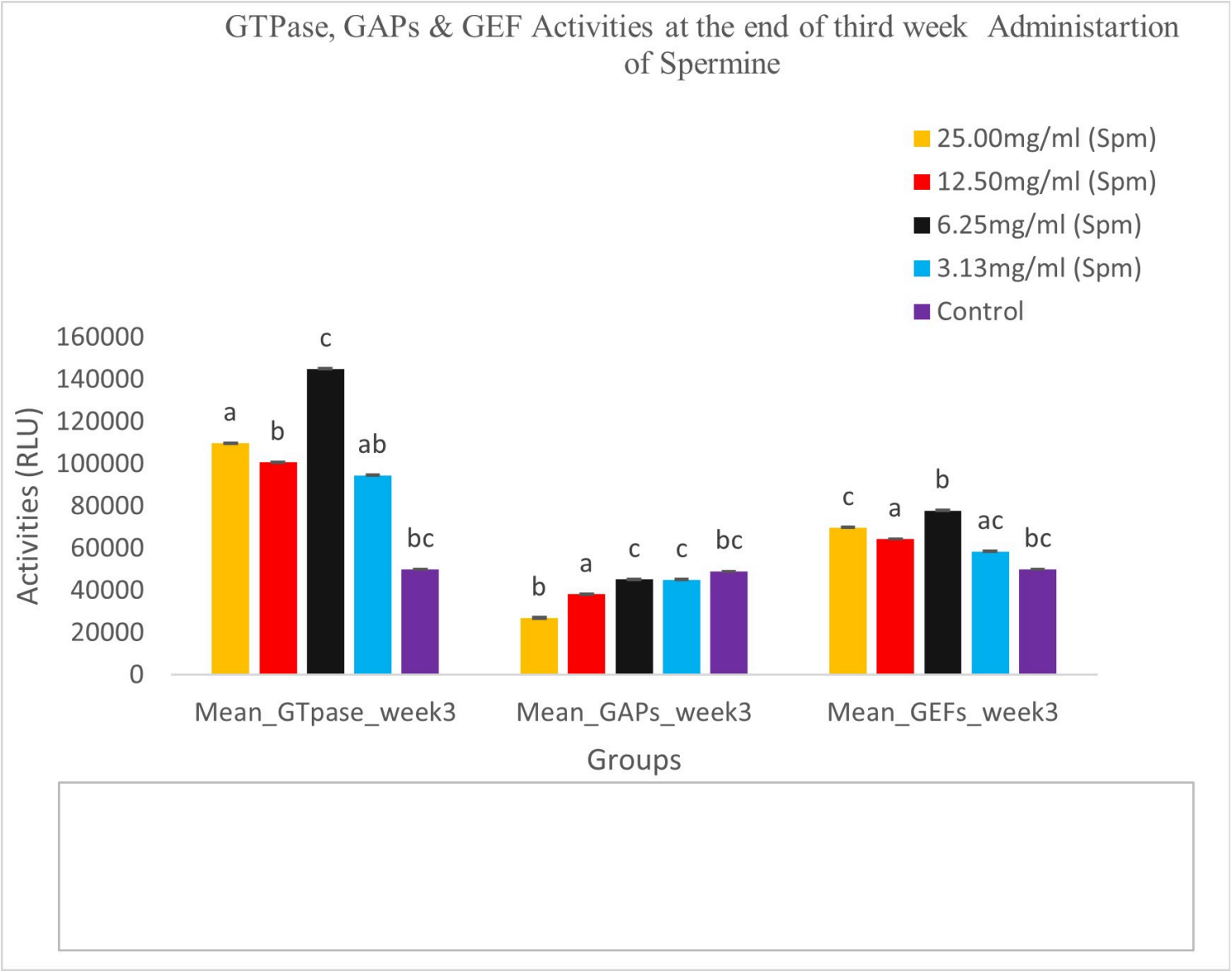


Figure 3

Ras GTPase activity, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factor (GEF) activities in GK rats after three weeks of spermine administration. Data are presented as mean ± standard error of the mean (SEM) for the measured parameters. Statistical significance was set at $p < 0.05$. *Spm* denotes spermine. Values sharing identical superscript letters are not significantly different.

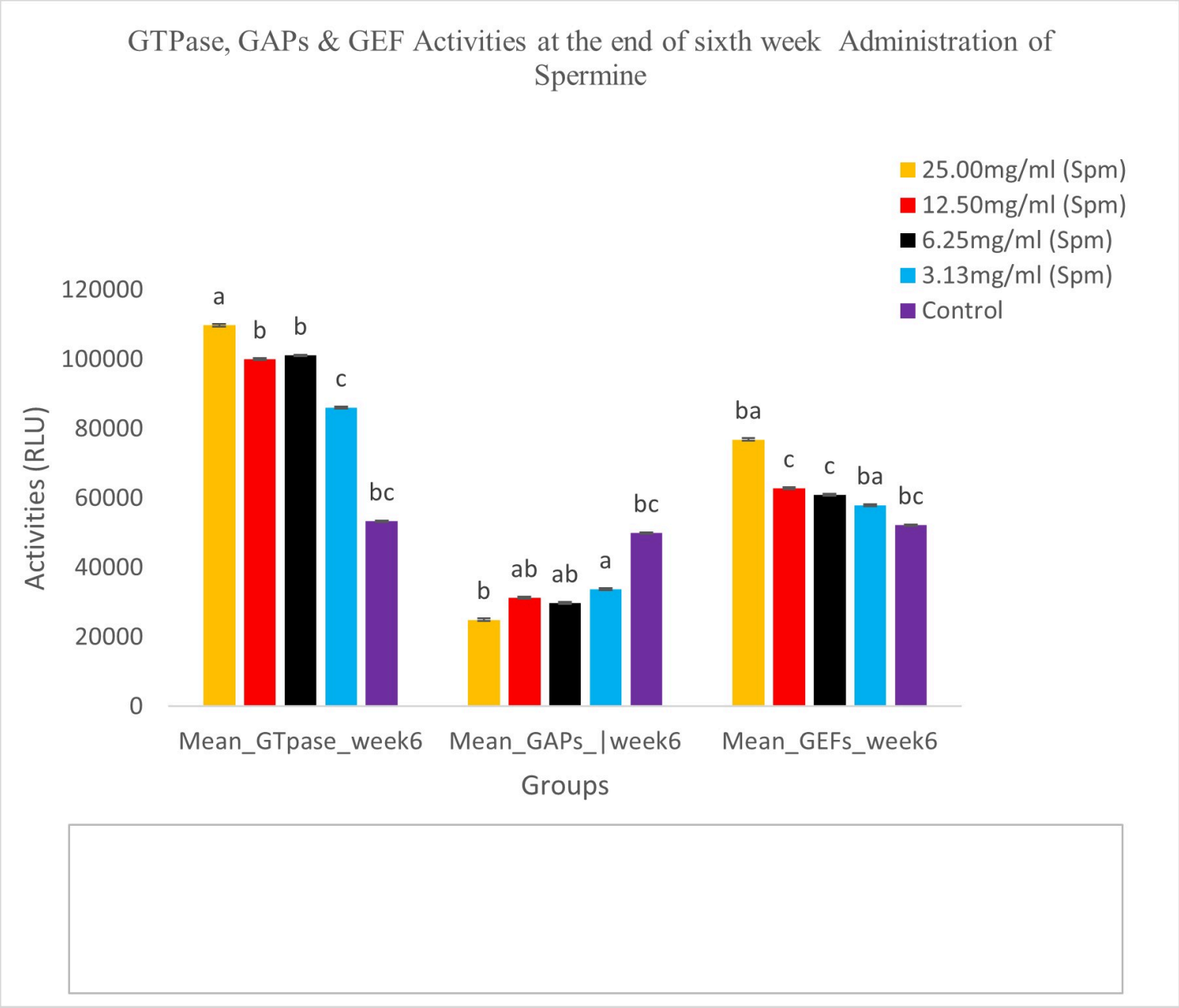


Figure 4

Illustrate the activities of Ras GTPase, GTPase-activating proteins (GAPs), and guanine nucleotide exchange factors (GEFs) in GK rats following six weeks of spermine (Spm) administration. Data are expressed as mean ± standard error of the mean (SEM), with statistical significance set at $p < 0.05$. Superscripts sharing identical letters indicate no statistically significant difference between groups.

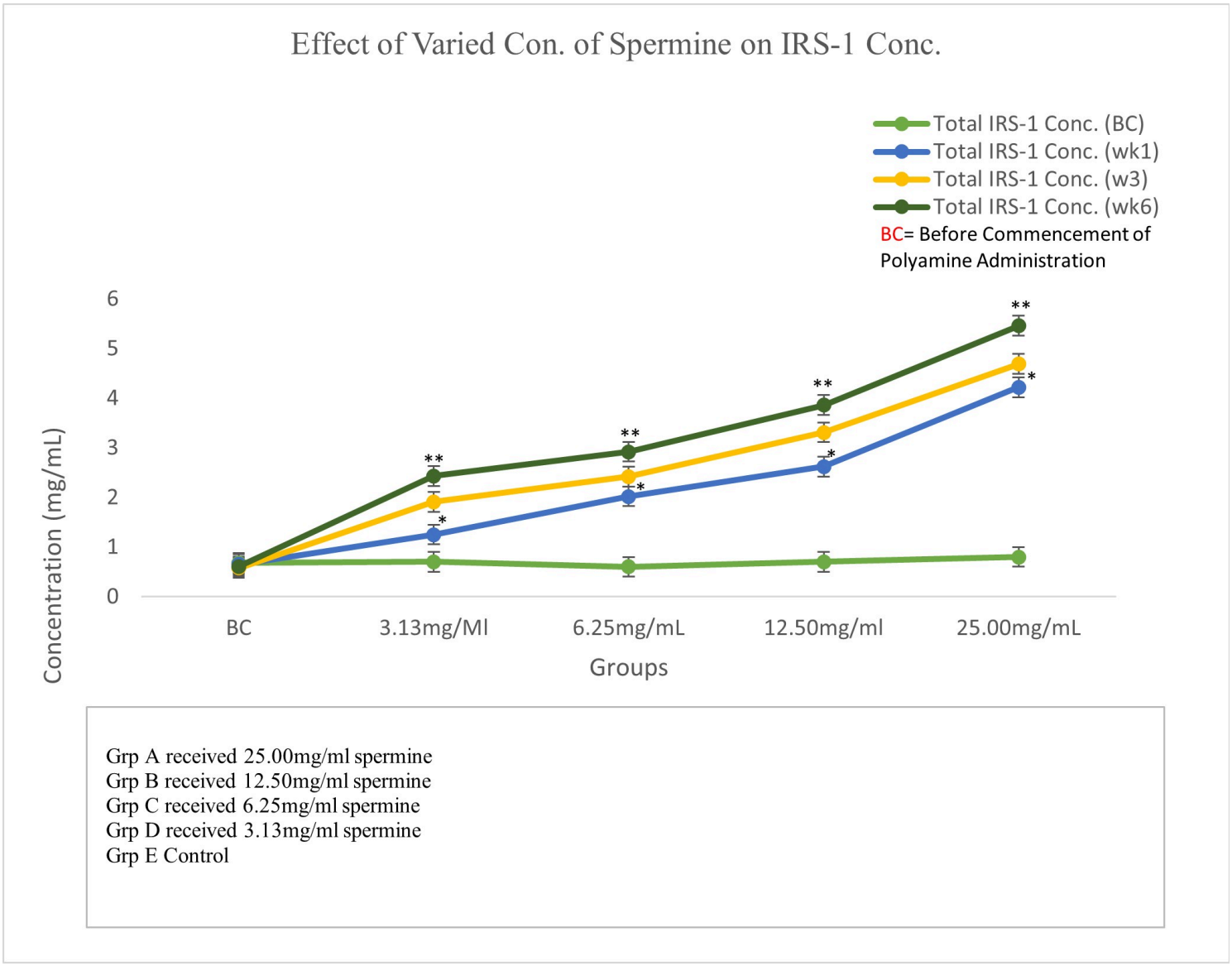
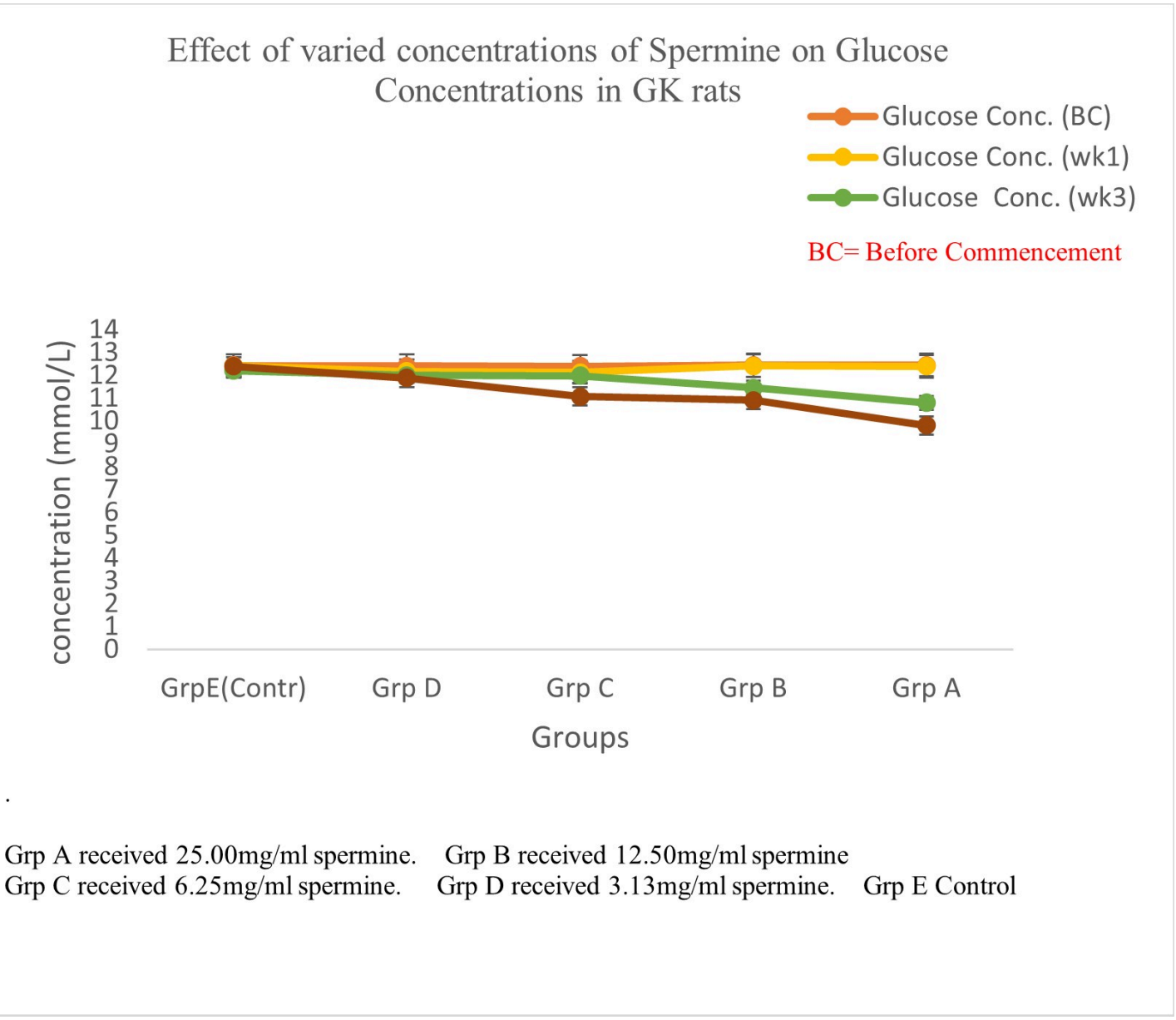


Figure 5

depicts total insulin receptor substrate-1 (IRS-1) concentrations in GK rats prior to spermine administration and at the end of weeks 1, 3, and 6 of treatment. Baseline values, denoted as (BC), represent measurements obtained before the initiation of spermine exposure.



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Grp A received 25.00mg/ml spermine. Grp B received 12.50mg/ml spermine
Grp C received 6.25mg/ml spermine. Grp D received 3.13mg/ml spermine. Grp E Control

Figure 6

Illustrates blood glucose concentrations in GK rats prior to the initiation of spermine administration and at the end of weeks 1, 3, and 6 of treatment. Baseline measurements, denoted as (BC), represent values obtained before spermine exposure