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The Investigation of Taurine on Muscle Myoblast Differentiation

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ABSTRACT:

AIM AND OBJECTIVE:

This research explores the potential of taurine supplementation to improve skeletal muscle differentiation, growth, and repair. The study primarily focuses on evaluating taurine's therapeutic role in addressing muscle-wasting conditions like sarcopenia and Duchenne Muscular Dystrophy (DMD). With the increasing prevalence of these conditions, particularly in aging populations, the study aims to assess taurine's ability to promote muscle cell proliferation and differentiation, while also determining the most effective concentration for maximizing its therapeutic benefits.

The main objective of this research is to assess the impact of taurine at different concentrations on skeletal muscle cells using an in vitro model. The study aims to determine the optimal concentration range of taurine that enhances muscle cell differentiation, fusion, and growth without inducing cytotoxic effects. It also investigates taurine's influence on critical differentiation markers, including the fusion index, myotube area, and aspect ratio. Additionally, the study explores how taurine affects the secretion of insulin-like growth factor binding proteins (IGFBPs) as a potential mechanism through which it supports muscle regeneration and repair.

METHODOLOGY:

Human skeletal muscle cells (C25) were cultured in vitro and exposed to varying concentrations of taurine (1 μM, 5 μM, 10 μM, 15 μM, 25 μM, 50 μM, 75 μM, and 100 μM). The WST-8 cytotoxicity assay was used to assess cell viability and identify any cytotoxic effects at higher taurine concentrations. Key markers of muscle differentiation, such as the fusion index, myotube area, and aspect ratio, were evaluated using immunofluorescence microscopy. Furthermore, an antibody microarray analysis was conducted

to investigate the secretion of insulin-like growth factor binding proteins (IGFBPs), which play a vital role in regulating muscle growth and repair.

RESULTS:

The WST-8 cytotoxicity assay showed that low to moderate taurine concentrations (1-50 μM) significantly improved muscle cell viability and differentiation, resulting in enhanced myotube formation, fusion, and alignment. This suggests taurine's potential in supporting muscle health. However, at a higher concentration of 100 μM, taurine induced cytotoxic effects, including cellular stress and reduced cell viability, likely due to oxidative stress. The antibody microarray analysis further indicated that moderate taurine levels (15-50 μM) increased the secretion of IGFBP1 and IGFBP3, both of which are involved in promoting muscle differentiation and cell survival. In contrast, at 100 μM, there was a notable rise in IGFBP1 and IGFBP6, signalling a stress response that could hinder muscle regeneration.

DISCUSSION:

The results of this study highlight taurine supplementation's potential to support skeletal muscle health when administered at moderate concentrations. Taurine demonstrated an ability to boost muscle differentiation, promote cell survival, and increase the secretion of IGFBPs, making it a promising therapeutic option for muscle-wasting conditions like sarcopenia and Duchenne Muscular Dystrophy (DMD). However, the study also stresses the need for accurate dosing, as higher taurine concentrations may cause cytotoxic effects and diminish its therapeutic value. Future research should prioritize clinical trials to confirm taurine's effectiveness and safety in addressing muscle-related disorders. Additionally, investigating the possible synergistic effects of taurine with other supplements or medications, such as antioxidants or calcium regulators, may help enhance its therapeutic impact. Considering the limitations of the in vitro model employed in this study, further in vivo research is essential to

fully explore taurine's clinical potential in improving muscle health in aging populations and those with muscular dystrophy.

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MSc BIOMEDICAL SCIENCE DISSERTATION DECLARATION 2024

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1.0 INTRODUCTION:

Skeletal muscles are among the most adaptable and multifunctional tissues in the human body, essential for enabling movement, maintaining posture, and regulating various metabolic processes. While primarily recognised for their role in voluntary movement, skeletal muscles are also integral to key physiological functions such as glucose uptake, protein storage, and the modulation of metabolic rate(Frontera and Ochala, 2015).Their distinctive structure, consisting of long, multinucleated fibres, equips them to perform these varied tasks effectively. Different types of muscles and their functions are as given (Figure 1)

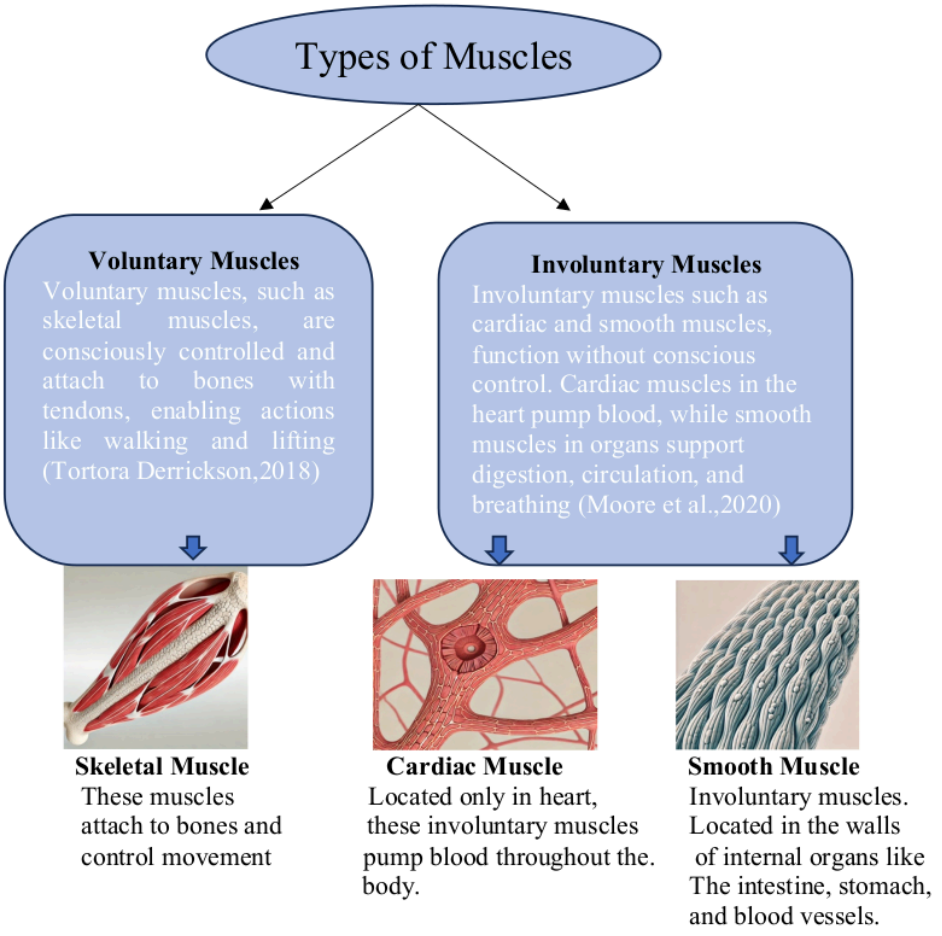

Figure.1 This figure depicts the three primary types of muscles in the human body: skeletal, cardiac, and smooth muscles. Each muscle type has specific roles and locations, playing a vital part in the body's movement, functioning, and overall health.

1.1 Functions of skeletal muscle

Skeletal muscle is composed of elongated, cylindrical cells known as muscle fibres. These fibres are grouped into bundles called fascicles, surrounded by three layers of connective tissue: the epimysium (encasing the entire muscle), perimysium (surrounding each fascicle), and endomysium (encasing individual fibres). Inside each muscle fibre are myofibrils, which are segmented into sarcomeres, the basic units of muscle contraction. Sarcomeres, made up of myosin (thick) and actin (thin) filaments, slide past each other during contraction, giving skeletal muscle its striated appearance (Figure 2). The primary role of skeletal muscle is to enable voluntary movements by contracting and exerting force on bones, supporting activities like walking, running, and lifting, as well as maintaining posture, stabilising joints, and generating heat during contraction (Hughes, 2003).

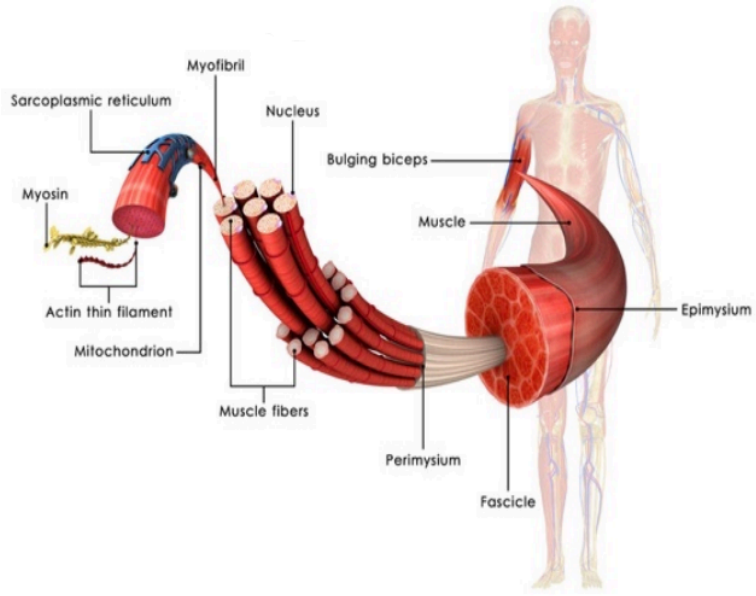

Figure 2 This diagram offers a comprehensive illustration of skeletal muscle structure, spanning from the macroscopic to the microscopic level. It emphasizes the essential components responsible for muscle contraction, including muscle fibres, fascicles, myofibrils, and molecular structures such as myosin and actin filaments, which facilitate the process of muscle contraction(Ólafsdóttir, 2017).

1.2 Muscle Proliferation, Differentiation and Myogenesis:

Myogenesis, the process of muscle formation, starts during embryonic development and continues throughout life (Figure 3). This involves the proliferation and differentiation of muscle precursor cells, or myoblasts, which fuse to form myotubes that develop into mature muscle fibres (Grefte *et al.*, 2007). Myogenesis is regulated by myogenic regulatory factors (MRFs), including MyoD, Myf5, myogenin, and MRF4, which are critical for muscle cell differentiation and growth (Buckingham and Rigby, 2014).

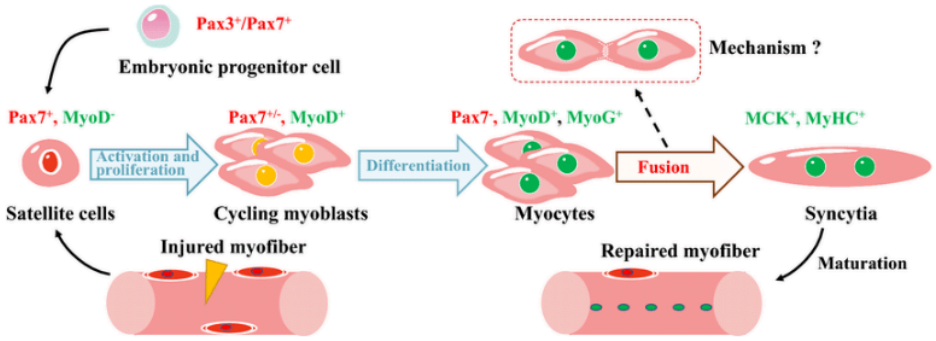

Figure 3 This figure demonstrates the Muscle Regeneration process, beginning with the activation of satellite cells and culminating in the development of mature muscle fibres (myofibers). It emphasizes the key steps, including the activation and proliferation of satellite cells, their transformation into cycling myoblasts, and the progression to myocyte formation. The diagram also shows the fusion of myocytes into syncytia, which then mature into repaired myofibers. Key molecular markers, such as Pax7, MyoD, MyoG, MCK, and MyHC, play a crucial role in regulating each phase of muscle regeneration. Additionally, a question is raised regarding the specific mechanism behind myocyte fusion during maturation.(Chen *et al.*, 2020).

Skeletal muscles are highly dynamic and versatile, playing key roles in movement, posture, and metabolic processes such as glucose uptake, protein storage, and metabolic rate regulation. Their unique structure, consisting of long, multinucleated fibres, enables these functions efficiently. Skeletal muscles are essential not only for movement but also for overall metabolic health. They are key sites for glucose absorption and utilisation, especially during and after exercise, which is vital for maintaining stable blood glucose levels and preventing conditions like diabetes(Turcotte and Fisher, 2008). Additionally, skeletal muscles act as a major storehouse for amino acids, which are vital for protein synthesis and energy generation. Their ability to store and release proteins makes skeletal muscles a crucial regulator of protein metabolism (Wolfe, 2006). Moreover, skeletal muscles significantly influence the body's basal metabolic rate (BMR), affecting total energy expenditure and fat oxidation(Zurlo *et al.*, 1990).

The regulation of these metabolic activities is closely related to muscle mass and fibre type, both of which can be affected by aging, exercise, and diseases such as Duchenne Muscular Dystrophy.

1.3 Formation of the Muscle and Regeneration:

Muscle formation, or myogenesis, is an ongoing process that occurs not only during normal growth and development but also in response to muscle damage. This regeneration is a highly intricate process that relies on satellite cells, which are specialized muscle stem cells located between the muscle fibre membrane (sarcolemma) and the basal lamina, the extracellular matrix that surrounds each muscle fibre (Yin, Price and Rudnicki, 2013). When muscle fibres are damaged due to injury, satellite cells are activated from their quiescent state. They then proliferate and differentiate into myoblasts, which are precursor cells that can fuse to form new muscle fibres or repair damaged ones (Relaix and Zammit, 2012).

This regenerative process is severely disrupted in pathological conditions like Duchenne Muscular Dystrophy (DMD). The muscle tissue undergoes repeated cycles of degeneration due to the lack of dystrophin, a protein critical for muscle fibre stability (Figure 4). The frequent damage leads to an overactivation of satellite cells, which eventually become exhausted and less effective. As a result, the muscle repair is incomplete, and the damaged fibres are progressively replaced by fibrotic tissue (scar tissue) and fat instead of new muscle tissue. This fibrosis and fatty infiltration contribute to the ongoing muscle weakness and functional decline characteristic of DMD(Emery, 2002). The inability to effectively regenerate muscle fibres result in a gradual loss of muscle function, severely impacting mobility and overall quality of life for affected individuals.

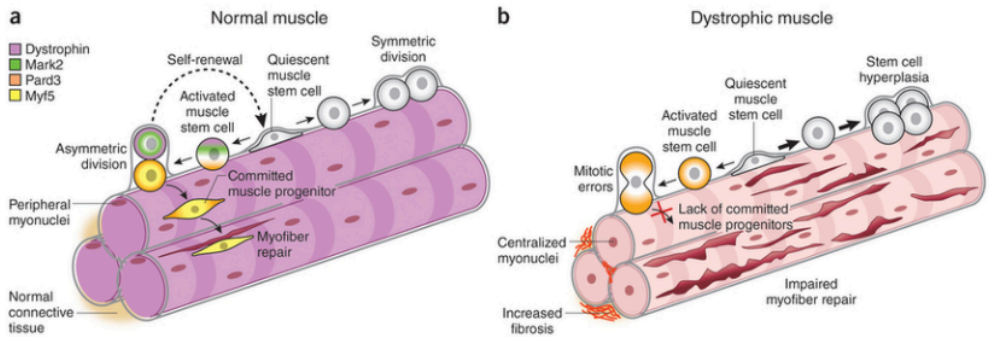

Figure.4 Normal Muscle Compared to Dystrophic Muscle(Keefe and Kardon, 2015)
(a) In normal muscle, dystrophin is present in activated satellite cells and myofibers. In satellite cells, dystrophin helps polarize Mark2 and Pard3 during cell division, leading to asymmetric divisions that produce both stem cells and committed muscle progenitors. In myofibers, dystrophin ensures muscle membrane integrity. (b) Dystrophin-null satellite cells lose Par-mediated cell polarity, causing errors in cell division and fewer asymmetric divisions. This reduction in differentiated myocytes leads to poor regeneration. Combined with the degeneration of dystrophin-null myofibers, this results in progressive muscle loss.

1.4 Muscle Ageing and Sarcopenia:

Muscle ageing, or sarcopenia, describes the progressive loss of muscle mass, strength, and function that happens naturally with age. This decline greatly affects the quality of life in older adults by impairing mobility, balance, and overall well-being (Cruz-Jentoft *et al.*, 2010). It is a major factor contributing to frailty, decreased mobility, and an increased risk of falls, fractures, and disability, with its onset typically beginning around age 50 and worsening with age, impacting quality of life and independence. The condition is caused by multiple factors, including reduced physical activity, hormonal changes, inflammation, and inadequate nutrition, which lead to a decrease in muscle protein synthesis and, consequently, muscle wasting (Dodds and Sayer, 2016).A reduced anabolic response to protein intake further accelerates muscle loss in older individuals (Paddon-Jones and Leidy, 2014). Sarcopenia involves a loss of fast-twitch (Type II) muscle fibres, impairing the ability to perform daily activities like standing up or climbing stairs, and is also linked to reduced mitochondrial function, lower satellite cell activity, and increased oxidative stress, all contributing to muscle degradation (Landi *et al.*, 2012). Diagnosing sarcopenia typically involves measuring muscle

mass and function through tools like DEXA and BIA scans, as well as strength and gait tests. Although there is no definitive cure, prevention and management strategies focus on regular resistance exercise and protein supplementation, both of which help slow muscle loss and improve function. Recent research also suggests that taurine could play a role in promoting muscle health and combating sarcopenia (Moore *et al.*, 2015).

Mechanisms of Muscle Ageing

1.4.1 Muscle Fibre Loss and Atrophy

With ageing, there is a reduction in both the quantity and size of muscle fibres, especially Type II (fast-twitch) fibres, which are more prone to atrophy. This decline results from various factors, such as decreased physical activity, hormonal shifts, and changes in muscle protein metabolism (Evans, 2010).

1.4.2 Reduced Satellite cell Function

Satellite cells, which are muscle stem cells crucial for repair and regeneration, diminish in both quantity and effectiveness as people age. This reduction in satellite cell function hampers muscle regeneration after injury or exercise, leading to a decrease in muscle mass and strength(Carlson and Conboy, 2007).

1.4.3 Mitochondrial Dysfunction

As muscles age, they show a reduction in mitochondrial function, characterised by lower ATP production, heightened oxidative stress, and a build-up of mitochondrial DNA mutations. These alterations lead to diminished muscle endurance and a greater tendency toward fatigue(Short *et al.*, 2005).

1.4.4 Altered Protein Synthesis and Degradation:

Ageing is linked to a decrease in muscle protein synthesis rates and an increase in protein breakdown. Maintaining muscle mass depends on the balance between these processes; therefore, a shift favouring protein degradation results in muscle wasting (Volpi *et al.*, 2001).

1.4.5 Chronic Inflammation:

Chronic low-grade inflammation, known as "inflammation," is a characteristic of ageing and is linked to higher levels of pro-inflammatory cytokines like TNF-α, IL-6, and CRP. This inflammatory condition can hinder muscle protein synthesis and encourage muscle breakdown, thereby exacerbating sarcopenia(Ferrucci *et al.*, 2005).

1.4.6 Neuromuscular Changes:

Ageing impacts the nervous system, causing the loss of motor neurons that connect to muscle fibres. This loss, or denervation, leads to muscle atrophy and reduced muscle strength. The deterioration in neuromuscular function is a key factor behind the reduced physical abilities seen in older adults (Deschenes, 2004).

1.5 Nutritional effects on Ageing of muscles:

As individuals age, the gradual decline in skeletal muscle mass, strength, and function—a condition known as sarcopenia—becomes a significant concern. This muscle deterioration not only impairs physical performance but also increases the risk of frailty, falls, and loss of independence. Addressing sarcopenia through proper nutrition plays a critical role in mitigating these effects by supporting muscle protein synthesis, reducing inflammation, and preserving muscle strength. Adequate protein intake is particularly vital for older adults, as it helps stimulate muscle protein synthesis and counteracts anabolic resistance, a condition where the body becomes less efficient at building muscle in response to dietary protein(Breen and Phillips, 2011). Ensuring sufficient protein consumption, particularly from high-quality sources like lean meats, dairy, and plant-based options, is essential for maintaining muscle mass. In addition to protein, other nutrients also play key roles in promoting muscle health. Vitamin D, for example, supports calcium absorption, which is crucial for muscle contraction and bone strength, making it an important factor in maintaining overall musculoskeletal health. Antioxidants, such as vitamins C and E, help combat oxidative stress, which can accelerate muscle ageing. These antioxidants work by neutralizing free radicals, thereby preventing muscle damage and promoting recovery. Omega-3 fatty acids, commonly found in fish oil, have anti-inflammatory properties that are beneficial for reducing chronic inflammation—a condition associated with age-related muscle loss. These fatty acids help support muscle function and improve protein synthesis. Creatine and taurine are also vital for muscle health. Creatine enhances muscle strength and endurance, while taurine aids in muscle preservation and repair, ensuring better overall muscle performance and recovery (Smith *et al.*, 2011). By incorporating these nutrients into a balanced diet, ageing adults can reduce their risk of sarcopenia and improve muscle function, strength, and overall health, helping to maintain their independence and quality of life as they age.

1.6 Malnutrition and ageing risk:

Malnutrition, characterized by insufficient intake or absorption of essential nutrients, seriously threats older adults' health. As individuals age, their nutritional requirements shift due to a combination of physiological, social, and psychological changes. The prevalence of malnutrition increases with advancing age, leading to declines in physical and cognitive abilities, heightened vulnerability to illnesses, and greater frailty. One major factor contributing to malnutrition in older adults is a reduction in appetite, commonly known as "anorexia of ageing." This condition stems from various causes such as diminished senses of taste and smell, slower digestion, and reduced feelings of hunger (Morley, 2017). Additionally, physical challenges like dental issues or difficulty swallowing exacerbate the problem by making it harder for older individuals to consume adequate food(Lesourd, 1997). Social factors, including isolation and depression, further heighten the risk by decreasing motivation to eat. Malnutrition plays a significant role in accelerating the ageing process, its contribution to sarcopenia - the gradual loss of muscle mass and strength, leading to physical frailty and decreased mobility. A lack of proper nutrition speeds up muscle protein degradation, reducing functional independence and raising the likelihood of falls and fractures (Robinson *et al.*, 2018). Moreover, malnourished older adults are more susceptible to chronic diseases like cardiovascular disease, diabetes, and osteoporosis, all of which can further diminish the quality of life and increase healthcare expenses. Additionally, malnutrition weakens the immune system, making elderly individuals more prone to infections and complicating their recovery from illnesses or surgeries (Norman *et al.*, 2008). This often creates a negative cycle, where illness worsens malnutrition, leading to further health deterioration. Cognitive decline is another concern, with evidence indicating that poor nutrition can contribute to the progression of conditions like dementia and other neurodegenerative diseases. Given the increased risk of malnutrition in the ageing population, it is vital to implement preventative measures such as routine nutritional screenings, education on balanced diets, and ensuring access to nutrient-rich foods(Morley, 2012). Research has shown that interventions like protein and energy supplementation, alongside vitamin D and calcium intake, can significantly enhance muscle strength and physical function in older adults(Bauer *et al.*, 2015).

1.7 TAURINE Supplementation and Muscle Health:

Taurine is a sulphur-containing amino acid that is highly prevalent in many human body tissues, especially in the heart, brain, retina, and skeletal muscles. Unlike typical amino acids, taurine does not get incorporated into proteins but is found in its free form within cells. It is essential for several physiological functions, such as the formation of bile salts, regulation of cellular water balance (osmoregulation), stabilisation of cell membranes, and calcium signalling (Schaffer and Kim, 2018).

Taurine is crucial for muscle health due to its involvement in several key functions. It regulates calcium homeostasis, ensuring proper muscle contraction by supporting the function of the sarcoplasmic reticulum, which manages calcium storage and release in muscle cells Taurine also possesses strong antioxidant properties that protect muscle cells from oxidative stress caused by reactive oxygen species (ROS) during intense exercise, helping to prevent muscle fatigue and maintain function (Schaffer and Kim, 2018). Additionally, taurine acts as an osmolyte, maintaining cell volume and fluid balance, which supports muscle endurance and reduces cramps during exercise(Huxtable, 1992). It also aids in muscle repair and regeneration by promoting the growth and differentiation of satellite cells, reducing inflammation, and minimising fibrosis (De Luca, Pierno and Camerino, 2015). Furthermore, taurine supplementation has been linked to enhanced exercise performance, improving endurance, reducing muscle damage, and accelerated recovery, likely due to its effects on calcium regulation, antioxidant defence, and osmoregulation (Militante and Lombardini, 2002).

1.8 AIM:

Given the increasing challenge of muscle dysfunction, such as sarcopenia, among individuals aged 50 and older, and the lack of a definitive therapy for this condition, this research seeks to investigate the effects of taurine, a nutrient supplement, on myogenic differentiation in human skeletal muscle stem cells using an in vitro model.

1.9 Objective:

This study aims to develop an in vitro human muscle model for experimental purposes, with a focus on investigating how different concentrations of a specific nutrient, taurine, affect muscle cell differentiation and function. To accomplish this, the following objectives have been identified:

- Culturing and Proliferation of Human Skeletal Muscle Cells (SkMC): Develop a culture of human skeletal muscle stem cells (C25 cell line) in a growth medium. Ensure optimal conditions for cell proliferation are consistently maintained, focusing on sustaining cell viability, and achieving a high confluence rate prior to initiating the differentiation process.
- Inducing Differentiation in Skeletal Muscle Cells: Shift the proliferated SkMCs from the growth medium to a differentiation medium to encourage the conversion of myoblasts into myotubes. Maintain stable and controlled conditions that facilitate the differentiation process, ensuring uniform cell development across all experimental groups.
- Nutrient Treatment and Varying Concentrations: Administer taurine to the differentiated muscle cells at five different concentrations (1 µM, 15 µM, 25 µM, 50 µM, and 100 µM). Assess the dose-dependent effects of taurine on muscle differentiation and cell functionality by examining cellular morphology and analysing specific differentiation markers.
- Cell Imaging and Quantitative Analysis: Obtain high-resolution images of the treated muscle cells through immunofluorescence microscopy. Evaluate essential differentiation metrics, including fusion index, myotube area, and aspect ratio, using image analysis tool ImageJ.
- Data Analysis and Identification of Optimal Taurine Concentration: Conduct statistical analysis on the gathered data to identify the taurine concentration that most effectively enhances muscle differentiation and function. Utilize statistical methods, including ANOVA and Tukey's post hoc tests, to compare treatment groups and pinpoint significant variations in cell behaviour and structural changes.

1.10 Project Hypothesis:

The primary aim of this research is to explore the potential effects of specific nutrient, particularly taurine, on the process of myogenic differentiation and the structural composition of skeletal muscles. This goal will be achieved by developing a novel, functional human muscle model. The research is guided by the following hypotheses:

- It is hypothesised that exposing skeletal muscle myoblasts to increasing concentrations of taurine may have a progressive positive effect on myogenic differentiation, particularly in relation to the formation of myotubes and the organisation and distribution of nuclei.
- Additionally, it is proposed that varying the quantities of this nutrients applied to muscle cells could influence the morphology and spatial arrangement of skeletal muscle fibres.

2 METHODOLOGY:

2.1 Skeletal Muscle stem culture:

For the experiment, a 25-year-old human skeletal muscle cell line (C25) was obtained in partnership with the Institute of Myology in Paris(Mamchaoui *et al.*, 2011). This non-commercial cell line has been immortalised. After thawing, the contents of a vial, which had been frozen and contained 1 ml (1 x 10^6) cells in a solution of 10% dimethyl sulfoxide and 90% fetal bovine serum, were transferred into a 9 ml conical tube. This tube had been pre-filled with a growth medium (GM) designed to promote the proliferation of skeletal muscle cells (SkMC), as specified in the Table 1.

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Table 1. Growth Media used for SkMC proliferation.

Growth Media Components	Concentrations
Dulbecco's standard modified eagles medium (Lonza, Bazylea, Switzerland)	60% (v/v)
Foetal Bovine Serum (Lonza, Bazylea, Switzerland)	20% (v/v)
Medium 199 with Earle's BSS (Lonza, Nottingham, UK)	20%
Antibiotic: Penicillin / Streptomycin (Sigma, UK)	1%
L- glutamine (Lonza, Nottingham, UK)	1%
Basic Fibroblast growth factor [bFGF] (Sigma-Aldrich, St. Louis, MO, USA)	1%
Fetunin from fatal bovine serum (Dorset, UK)	25 µg/ml
Dexamethasone from Sigma-Aldrich (Dorset, UK)	5 µg/ml

2.2 Proliferation and cell counting:

The 10 ml suspension of the C25 cell line was transferred into T75 flasks and incubated at 37°C in an atmosphere containing 5% CO2.The experiment continued until the cells reached 80% confluence. Once the flasks reached 80% confluence, the growth medium (GM) was removed, and the cells were washed twice with Dulbecco's Phosphate-Buffered Saline (DPBS). Then, 2 ml of TrypLE™ Express Enzyme was added to dissociate the cells, followed by incubation at 37°C in a 5% CO2 environment for 5 minutes. The dissociated cells, now in a 2 ml suspension, were transferred to a conical tube and mixed with 10 ml of growth medium (GM). For cell counting, a 50µl sample of Trypan Blue Stain (0.4%) was mixed with an equal volume of the cell suspension, and the number of viable cells was counted using a haemocytometer (Figure 5). The concentration of live cells per millilitre was calculated using the formula: [The average count of viable cells in one large corner square, multiplied by the dilution factor, and then multiplied by 10^6].

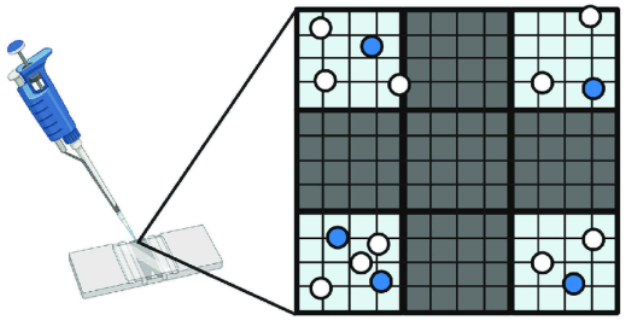

Figure 5 : Picture of haemocytometer with the enlarger chamber containing live cells(clear) and apoptotic cells(blue)(Pioli and Pioli, 2023).

2.3 Cytotoxicity Assay or WST8 Assay:

This cytotoxicity assay was designed to assess the impact of taurine on the viability and cytotoxicity of skeletal muscle cells using the WST-8 reagent, which offers a colorimetric assessment of cell viability. The experiment involved preparing various taurine concentrations, treating the cells, and evaluating cytotoxicity by measuring absorbance at 460 nm. The assay was carried out using a 96-well tissue culture plate with a clear bottom.

2.3.1 Cell Seeding:

15,000 cells were seeded per well in 100 µl of growth medium (GM) into two 96-well tissue culture plates with clear bottoms. The plates were then incubated at 37°C in a 5% CO2 incubator for 24 hours to allow the cells to adhere and stabilize.

2.3.2 Preparation of the nutrient:

In this experiment, taurine was used as the nutrient to assess its effectiveness in promoting the growth of skeletal muscle stem cells. The amount of nutrient was calculated by considering its molecular weight, the mass of each solute, and the desired concentration. Once the total solution volume was established, the appropriate solvent, DMSO (Dimethyl sulfoxide), was added accordingly. This process involved analysing the solubility of the nutrient, referencing details such as the name, code, and Lot number provided on the packaging. These details were entered into the manufacturer's website to obtain the necessary information. The nutrient was then dissolved in DMSO, and the Eppendorf tubes were labelled according to the specific concentrations used for testing, which were 1 µM, 5 µM, 10 µM, 15 µM, 25 µM, 50 µM, 75 µM and 100 µM.

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2.3.3 Pre-treatment Incubation:

After a 24-hour incubation, the growth medium (GM) was carefully removed from each well, and the cultured cells were washed twice with 1 ml of phosphate-buffered saline (PBS) per well to remove any residual medium. Then, 100 µl of differentiation medium (DM) was added to each well, and the plates were incubated for an additional 48 hours at 37°C in a 5% CO2 incubator(Freshney, 2015).The components of Differentiation media that was used in the experiment is as in the Table 2.

Table 2: Components of Differentiation Medium (DM)

Differentiation Media Components	Concentration
Dulbecco's Standard Modified Eagles Medium (Lonza, Bazylea,Switzerland)	500ml
L-glutamine (Lonza, Nottingham, UK)	1%(v/v)
Antibiotic: Penicillin / Streptomycin (Sigma, UK)	1%(v/v)

2.3.4 Taurine treatment:

After the 48-hour incubation period, the differentiation medium (DM) was removed from each well, and the wells were washed twice with PBS. Then, 100 µl of the prepared taurine solutions, with concentrations ranging from 1 µM to 100 µM, were added to the appropriate wells in the 96-well plate. For the control wells, DM without any treatment was added, and for the blank wells, medium without any cells was added. The plates were incubated for another 48 hours at 37°C in a 5% CO2 incubator.

2.3.5 Cytotoxicity Assay:

After 48 hours of incubation with the treatment, the cells were examined under an inverted microscope to detect any morphological changes or signs of cytotoxicity, such as cell rounding, detachment, or reduced cell density. To assess cell viability, 10 µl of WST-8 reagent was added to each well. This reagent interacted with metabolically active cells, producing a measurable colorimetric or fluorometric signal. The plates were then incubated in the dark for 1 to 2 hours at 37°C to allow sufficient time for the reagent to interact with the cells and develop a detectable signal(Berridge and Tan, 1993).

2.3.6 Measurement of Absorbance:

The absorbance was measured at 460 nm using a microplate reader at both 1-hour and 2-hour intervals to quantify the metabolic activity of the cells. The absorbance was directly proportional to the number of viable cells, reflecting the cytotoxic effect of taurine at various concentrations.

2.3.7 Data Analysis:

Cell viability was calculated as a percentage relative to the control (untreated cells) to evaluate the cytotoxic effects of the different taurine concentrations. The data were analysed to identify the concentration at which taurine showed cytotoxicity, if any, and to assess the dose-response relationship.

Higher absorbance values indicate greater cell viability and lower cytotoxicity, implying that the cells have enhanced metabolic activity and overall health. Conversely, lower absorbance values suggest reduced cell viability and potential cytotoxic effects. The Optical Density (OD) values for the treatment with Taurine was calculated after 1hour at 460nm.

Based on the cytotoxicity assay data, the five concentrations—1 µM, 15 µM, 25 µM, 50 µM, and 100 µM—were chosen for further analysis due to their impact on cell viability, as reflected in the percentage viability (% Viability) values. These concentrations were selected to represent a range of effects, from minimal to significant changes in cell viability, and to highlight those that showed noticeable differences in viability compared to the control. The selected concentrations ranged from low to high taurine levels, allowing for a thorough evaluation of the dose-response relationship and potential cytotoxic effects. This selection was intended to provide insights into the concentration ranges where taurine might have been beneficial, neutral, or potentially harmful based on the observed cell viability.

In this experiment, 6-well plates coated with a 0.5% gelatine solution were used, and each millilitre of growth medium (GM) was seeded with 1.5 x 10^5 C25 skeletal muscle cells (SkMC). The cell cultures were then incubated at 37°C in an environment containing 5% carbon dioxide (CO2) for a period not exceeding 24 hours, under strictly controlled conditions. After the incubation period, the growth medium was removed, and the cells were washed three times with Dulbecco's Phosphate-Buffered Saline (DPBS). Following the washing, 2 ml of differentiation medium (DM) was added to each well, and the plates were incubated again for 72 hours at 37°C with 5% CO2.

2.4 SkMC Differentiation:

C25 cells were seeded in a T75 flask and incubated at 37°C for 4 to 5 days until they reached confluence. A 1% gelatine solution was prepared and maintained at 37°C. The gelatine solution was then sterilized and added to each well of a 6-well plate. The P8 flasks containing C25 cells were removed from the incubator, and the media was discarded. The flasks were washed twice with sterilized phosphate-buffered saline (PBS) to eliminate dead cells and serum residues, ensuring the triple E enzyme would function properly. Then, 1.5 ml of the triple E enzyme was added to each flask and incubated for 10 minutes. The suspension culture was monitored under a microscope. To neutralize the enzyme's activity, 2 ml of medium was added to each flask. The contents were then transferred to tubes, and an additional amount of medium was added to the flasks and transferred to ensure all cells were collected. The tubes were vortexed to mix the contents thoroughly. A 50 µl sample of the culture from P8 was taken into an Eppendorf tube, and the remaining culture was incubated. To the Eppendorf tubes, 50 µl of trypan blue was added, and the tubes were vortexed. The cell count was determined using a haemocytometer. The total number of cells needed was calculated using the formula: total cells required = desired concentration multiplied by total volume, i.e., 150,000 cells/ml multiplied by 13 ml, resulting in a total of 195,000 cells. The dilution equation C1V1 = C2V2 was used, where C1 is the concentration of the original suspension, V1 is the volume needed, C2 is the desired concentration, and V2 is the total volume. The total required volume was 13 ml. If 1.2 ml of the original suspension was used, the amount of diluent needed would be 13 ml - 1.2 ml = 11.8 ml. Therefore, 1.2 ml of the original cell suspension (1.64 x 10^6 cells/ml) was mixed with 11.8 ml of diluent to achieve a final concentration of 150,000 cells/ml in a total volume of 13 ml. 2 ml of this mixture was distributed into each of the 6 wells containing gelatine. The well plates were then incubated for 48 hours at 37°C in a 5% CO2 incubator.

2.4.1 Fixation using Formaldehyde:

Gently remove the medium from each well of the 6-well plate. Rinse each well twice with 1 ml of PBS per wash to eliminate any remaining medium. Add 1 ml of formaldehyde solution (typically 4% in PBS) to each well and incubate the plate at room temperature for 15 minutes.

2.4.2 Permeabilization:

To prepare the permeabilization solution, mix 5 µl of a 0.5% Triton X-100 solution with 10 ml of PBS. After incubating the cells with formaldehyde for 15 minutes, carefully remove the solution from each well. Rinse each well twice with 1 ml of PBS to ensure any remaining formaldehyde is washed away. Then, add 1 ml of the 0.5% Triton X-100 solution to each well and incubate at room temperature for another 15 minutes. Following this, gently remove the Triton X-100 solution from each well and wash each well twice with PBS.

2.4.3 Blocking with Bovine Serum:

Add 2 ml of 5% Bovine Serum Albumin (BSA) to each well and incubate the plate on a shaker at room temperature for 1 hour to block non-specific binding sites.

2.5 Staining:

2.5.1 Preparation of the stains:

Preparation of DAPI Stain(4′,6-Diamidino-2-phenylindole): To prepare a 1:10,000 dilution of DAPI, mix 1 µl of DAPI with 10 µl of PBS.

Preparation of Myosin Heavy Chain Stain (Green Stain): To prepare a 1:400 dilution, add 5.2 µl of the Myosin Heavy Chain stain to 2 ml of the prepared DAPI solution.

2.5.2. Staining Procedure

After a 1-hour incubation with Bovine Serum Albumin (BSA), carefully remove the BSA solution from each well and wash each well once with PBS to remove any remaining BSA. Next, add 300 µl of the prepared staining solution to each well and incubate the plate at room temperature for the appropriate duration, typically ranging from 30 minutes to 1 hour. Following incubation, gently remove the staining solution from each well and wash the wells twice with PBS to eliminate any unbound stain. Finally, add 1 ml of PBS to each well to prevent the cells from drying out during imaging. Image analysis was done using ImageJ Software(Schneider, Rasband and Eliceiri, 2012).

2.6 Differentiation Parameters:

The fusion index percentage is calculated using the formula: (number of nuclei within multinucleated cells / total number of nuclei in a specific field) * 100. This index is commonly employed in muscle cell culture studies to measure the degree of myoblast fusion. The myotube area percentage is calculated using the formula: (myotube area / field area) * 100. Myotubes are defined as elongated, tube-shaped structures, formed by the fusion of myoblasts into a syncytium. This specific morphology, identifiable through MyHC antibody staining, is a hallmark of myotubes. Additionally, myotubes are characterized by the presence of at least two nuclei, which is one of their distinguishing features. The aspect ratio is determined by dividing the myotube length by the myotube width. The aspect ratio (AR), which represents the ratio of the short axis to the long axis of a myotube, has been demonstrated to correlate with both differentiation and contractile properties(Chamley-Campbell, Campbell and Ross, 1979).

Differentiation Parameters	Formula
Fusion Index% (FI%)	[Total number of nuclei per myotube/Total number of nuclei in the field] *100
Myotube Area% (MA%)	[Total area of Myotubes in a field/Total area of the field] *100
Aspect Ratio (AR)	Myotube Length / Myotube Width

2.7 Image Analysis using ImageJ:

The ImageJ program is recognized as one of the first open-source tools for analysing scientific images. Images taken with a Leica TCS SP5 confocal microscope (Leica Microsystems, Wetzlar, Germany) were analysed using ImageJ in this study. The experiment utilized the six well plate containing various concentrations of Taurine. The well plate contained a control well without Taurine. Five images were taken for each well, capturing different angles such as top, bottom, centre, right, and left, including the control wells. In total, thirty images of wells treated with taurine (including a control well) were taken and analysed. The ImageJ program itself does not directly perform measurements or data analysis but provides tools to quantify

parameters such as the dimensions (length and width) and the number of nuclei in myotubes (Figure 6). The collected data is then transferred to an Excel spreadsheet for further analysis. Before measuring the myotubes, a scale is set for each image, standardized at 2 pixels/µm, which serves as a global scale across all images. The ROI (Region of Interest) manager in ImageJ provides measured data such as area, mean, and angle. This method was used to analyse all the relevant parameters.

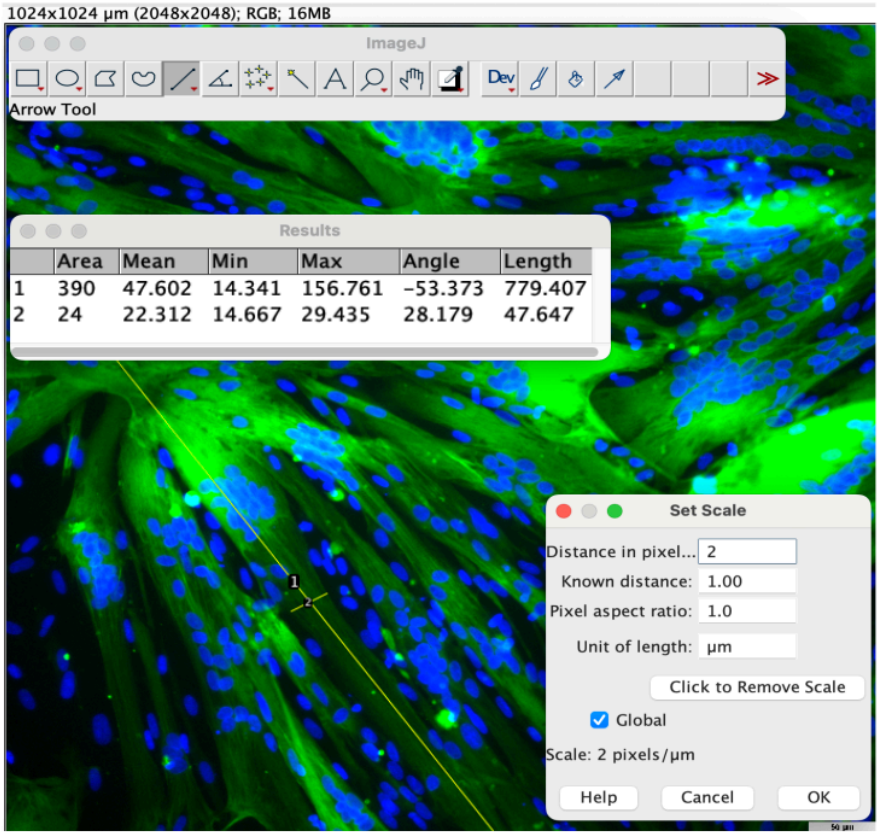

Figure 6 This image illustrates the analysis of skeletal muscle cells using immunofluorescence microscopy within the ImageJ software. The green-stained regions represent myotubes, while the blue staining indicates nuclei. The "Set Scale" box is used to calibrate the image, with 2 pixels corresponding to 1 µm. The "Results" table provides measurements of selected regions of interest (ROIs), detailing parameters such as area, average intensity, minimum and maximum intensity, angle, and myotube length. These values are used to assess important differentiation metrics, including the size and length of the myotubes.

For the analysis of immunofluorescent images of myotubes, photos were taken using a Leica TCS SP5 Confocal microscope. Five representative fields of view were randomly selected for analysis. The "ROI Manager" tool in ImageJ (found under the "Analyse → Tools" menu) was used to measure the length and width of the myotubes consistently across all images. Additionally, the "analyse particle" function was employed to set the scale based on the specified pixel size (in pixel^2).

2.8 Micro- Array:

In this experiment, an antibody array was utilised to simultaneously detect 12 growth factors. C25 skeletal muscle cells were cultured and exposed to different concentrations of taurine (1 µM, 15 µM, 25 µM, 50 µM, and 100 µM). After the incubation period, supernatant samples were gathered for further analysis. The microarray analysis assessed the secretion of various growth factors and insulin-like growth factor binding proteins (IGFBPs) in response to different concentrations of taurine in C25 skeletal muscle cells.

2.8.1 Preparation of Antibody Array Membranes:

The antibody array membranes (C1), pre-coated with specific antibodies, were prepared for incubation using the following steps: The membranes were first blocked with blocking buffer and left to incubate for 30 minutes at room temperature (RT)to minimise non-specific binding. After this step, the blocking buffer was removed, and 1 ml of each sample (either diluted or undiluted), along with standards and control, was applied to the membranes. The membranes were then incubated overnight at 4ºC, allowing the growth factors in the samples to bind to the immobilised antibodies. The Proteins tested are as given in the table below (Table 3)

Table 3. List of proteins tested in Microarray.

Protein Tested	Description
GDF-15	Growth/differentiation factor 15
GDNF	Glial cell line derived neurotrophic factor
GH	Somatotropin (GH)
HB-EGF	Proheparin -binding EGF- Like growth factor
HGF	Hepatocyte growth factor
IGFBP-1	Insulin like growth factor binding protein 1
IGFBP-2	Insulin like growth factor binding protein 2
IGFBP-3	Insulin like growth factor binding protein 3
IGFBP-4	Insulin like growth factor binding protein 4
IGFBP-6	Insulin like growth factor binding protein 6
IGF-1	Insulin like growth factor 1
Insulin	Insulin

2.8.2 Washing and Antibody Detection:

After incubation, the membranes underwent a series of wash steps: The samples were aspirated, and the membranes were washed three times with 2 ml of wash buffer 1 to eliminate any unbound proteins. A final wash was carried out with 2 ml of wash buffer 2 to ensure complete cleaning. Next, 1 ml of biotinylated antibody cocktail was applied to each membrane and left to incubate for 1.5 hours at room temperature. Once the incubation was complete, the antibody cocktail was removed, and 2 ml of single-use HRP-streptavidin was added to each membrane and incubated for an additional 2 hours at RT.

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2.8.3 Imaging:

The prepared membranes were then exposed to a chemiluminescence imaging device, such as a CCD camera, to capture the signals from the growth factors bound to the array. This approach allowed for the simultaneous detection of 12 growth factors, providing essential data to evaluate the effects of taurine on C25 skeletal muscle cells.

2.9 Statistical Analysis:

The research utilised GraphPad Prism version 10.0, a statistical software developed by GraphPad Software Inc., based in La Jolla, California, USA. The study employed statistical methods to compare averages across multiple groups. A one-way analysis of variance (ANOVA) was performed to determine overall differences between group means. To identify specific pairwise differences among the means, Tukey's multiple comparisons test was conducted as a post hoc analysis. Statistical significance was denoted by asterisks, with *() indicating $p \leq 0.05$, ()* ***p ≤ 0.01, ()*** $p \leq 0.001$, and (****) $p \leq 0.0001$. Results were expressed as mean values with standard deviations (mean ± SD), and the threshold for statistical significance was set at $p < 0.05$.

3 RESULTS:

3.1 Result Outline:

The aim of the research was to identify which nutritional supplement, within safe dosage levels, would most effectively promote muscle development. In this study, human skeletal muscle stem cells were cultured in vitro under controlled conditions. Throughout the experiment, these cells underwent proliferation and differentiation. Subsequently, they were treated with the nutrient, taurine at varying concentrations of 1 μM, 15 μM, 25 μM, 50 μM, and 100 μM. The growth of the cells was assessed by comparing them to a control well containing only differentiation medium and stem cells, without any added nutrients, which served as the reference standard. The results indicated positive effects as nutrient concentrations increased, such as enhanced differentiation parameters. There was a notable increase in the myotube area, fusion index, and aspect ratio.

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3.2 Cytotoxicity Assay:

The WST-8 cytotoxicity assay performed after 1 hour of taurine treatment at different concentrations (1 µM, 5 µM, 10 µM, 15 µM, 25 µM, 50 µM, 75 µM, and 100 µM) showed that cell viability increased as taurine levels rose. The control group, which received no taurine, maintained a 100% viability baseline. At the lower taurine concentrations (1 µM, 5 µM, and 10 µM), slight increases in viability were observed, with percentages of 101.64%, 102.32%, and 108.82%, respectively. The OD values obtained after 1 hour is as below (Table 4)

	Control	**1 µM**	**5 µM**	**10 µM**	**15 µM**	**25 µM**	**50 µM**	**75 µM**	**100µM**
Mean	**0.38155**	**0.3878**	**0.3904**	**0.4152**	**0.4218**	**0.4224**	**0.4312**	**0.4662**	**0.4786**
%Viability	**100**	**101.638055**	**102.319486**	**108.8193**	**110.5941**	**110.7063**	**113.0127**	**122.1858**	**125.4357**
Standard Deviation	**0.0296577**	**0.00969536**	**0.01184483**	**0.011149**	**0.018152**	**0.037892**	**0.049918**	**0.020971**	**0.01862**
Viability% Control	**100**	**1001**	**100**	**100**	**100**	**100**	**100**	**100**	**100**

Table 4 The Optical Density (OD) values recorded after the first hour of a cytotoxicity assay performed on C25 skeletal muscle cells exposed to various concentrations of taurine (1 µM, 5 µM, 10 µM, 15 µM, 25 µM, 50 µM, 75 µM, and 100 µM). These OD values, measured at 460 nm, indicate cell viability, with higher readings reflecting increased metabolic activity and enhanced cell viability. The control group, which did not receive taurine treatment, is used as the baseline, representing 100% viability. As taurine concentration increases, a corresponding rise in cell viability is observed, reaching its highest point at 100 µM, where early signs of cytotoxicity are noted, as suggested by the elevated OD values. The figure illustrates a dose-dependent effect, showing taurine's influence on cell viability and potential cytotoxicity across different concentrations.

As the taurine concentration increased, so did cell viability. At 15 µM and 25 µM, viability rose to 110.59% and 110.71%, reflecting a moderate enhancement of cell health. The highest taurine concentrations (50 µM, 75 µM, and 100 µM) led to even greater viability increases, reaching 113.01%, 122.19%, and 125.44%. However, at these higher concentrations, signs of cytotoxicity began to emerge, as shown by the increasing standard deviation values at 50 µM (0.0499), 75 µM (0.021), and 100 µM (0.0186). This variation suggests that while some cells responded positively to taurine, others may have experienced adverse effects, indicating potential cytotoxicity at higher concentrations (figure 7).

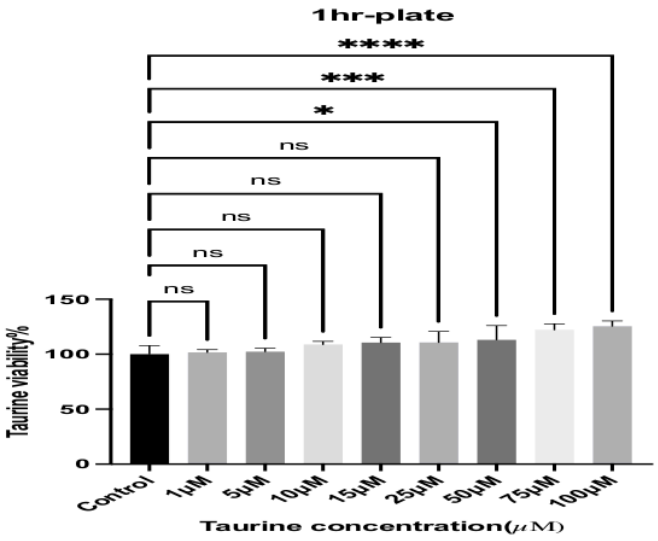

Figure 7. Cytotoxicity Results of C25 Skeletal Muscle Cells Treated with Taurine for 1 Hour at Various Concentrations (WST-8 Assay). The x-axis indicates the taurine

concentrations (1 µM, 5 µM, 10 µM, 15 µM, 25 µM, 50 µM, 75 µM, and 100 µM), while the y-axis represents the OD values, which are linked to cell viability. As taurine concentration rises, OD values also increase, signifying improved cell viability and metabolic activity, with the highest value observed at 100 µM. The control bar, which represents untreated cells, serves as a reference point.

Given these results, the second hour of testing was not performed to avoid further cytotoxic damage. The WST-8 assay effectively assesses cell viability, and the findings indicate that while taurine can enhance viability at lower to moderate levels, higher concentrations may lead to harmful cytotoxic effect(Flora, Shrivastava and Mittal, 2013).

3.3 SkMC cell Culture Treated with Taurine:

The data illustrated in the graphs (Figure 5) show notable changes in the differentiation parameters of skeletal muscle cells exposed to varying concentrations of taurine.

Fusion Index: The fusion index, which indicates the degree of myoblast fusion into multinucleated myotubes, increases significantly with higher concentrations of taurine, especially at 15 µM, 25 µM, and 50 µM, compared to the control group. The statistical significance of these increases is denoted by asterisks, with particularly significant rises observed at 25 µM and 50 µM concentrations ($*p \leq 0.05$) (Figure 8 – a).

Myotube Area: The overall size of the myotubes, as indicated by the myotube area, showed a significant increase with rising taurine concentrations. This enhancement was most pronounced at concentrations of 25 µM, 50 µM, and 100µM, suggesting that taurine facilitates muscle growth and hypertrophy. These changes were statistically significant at concentrations of 25 µM and higher, with asterisks marking the significance levels ($*p \leq 0.05$, $**p \leq 0.01$) (Figure 8 – b)

Aspect Ratio: The aspect ratio, which indicates the elongation and development of myotubes, also displayed a pattern of increase with higher levels of taurine. The most notable improvements were seen at 25 µM and 50 µM, suggesting enhanced myotube alignment and differentiation. These increases were statistically significant, as highlighted by the asterisks ($*p \leq 0.05$) (Figure 8 – c).

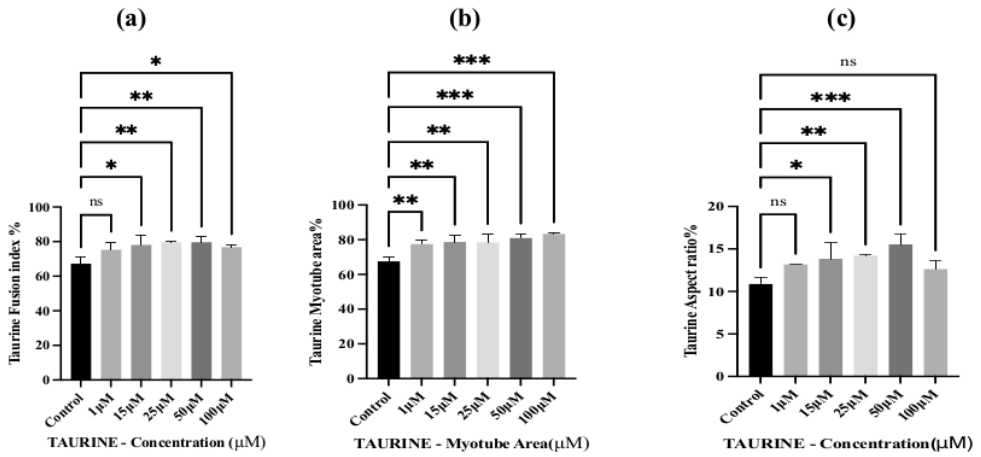

Figure 8 These figures display bar graphs showing the cytotoxicity assay results for skeletal muscle cells treated with different taurine concentrations. The x-axis represents taurine levels, and the y-axis indicates Optical Density (OD) values, reflecting cell viability. (a) Cell viability increases with taurine concentration, suggesting enhanced muscle cell health up to a certain point. The control group, without taurine, serves as a baseline. (b) The graphs highlight a dose-dependent effect, with cell viability peaking at moderate taurine levels. Higher concentrations show reduced viability, indicating potential cytotoxicity beyond a certain threshold.

The findings indicate that taurine supplementation promotes skeletal muscle differentiation, evidenced by significant increases in fusion index, myotube area, and aspect ratio, especially at concentrations ranging from 15 µM to 100 µM. The statistically significant changes, marked by strikes and asterisks, underscore taurine's potential as a beneficial nutrient for enhancing muscle cell growth and differentiation(Collett-Solberg and Cohen, 1996).

These images (figure 9) display C25 skeletal muscle cells treated with varying taurine concentrations, stained with DAPI (blue) to visualize nuclei and myosin (green) to highlight myotube formation. The differences in myotube formation, fusion, and alignment suggest that taurine concentration influences muscle cell behaviour. The control image, without taurine, shows normal myotube alignment and elongation, indicating typical differentiation. At 1 µM taurine, there is a slight enhancement in cell alignment and elongation, suggesting early differentiation benefits. Moderate concentrations (15-50 µM) exhibit significantly improved myotube formation and alignment, with optimal differentiation and fusion seen at 50 µM. In contrast, the highest concentration (100 µM) shows signs of cellular stress and overcrowding, implying potential cytotoxicity despite maintained alignment and fusion. Overall, taurine concentrations of 15-50 µM promote myotube formation and muscle health, while higher

concentrations (100 µM) may negatively impact cell viability and function due to cytotoxic effects.

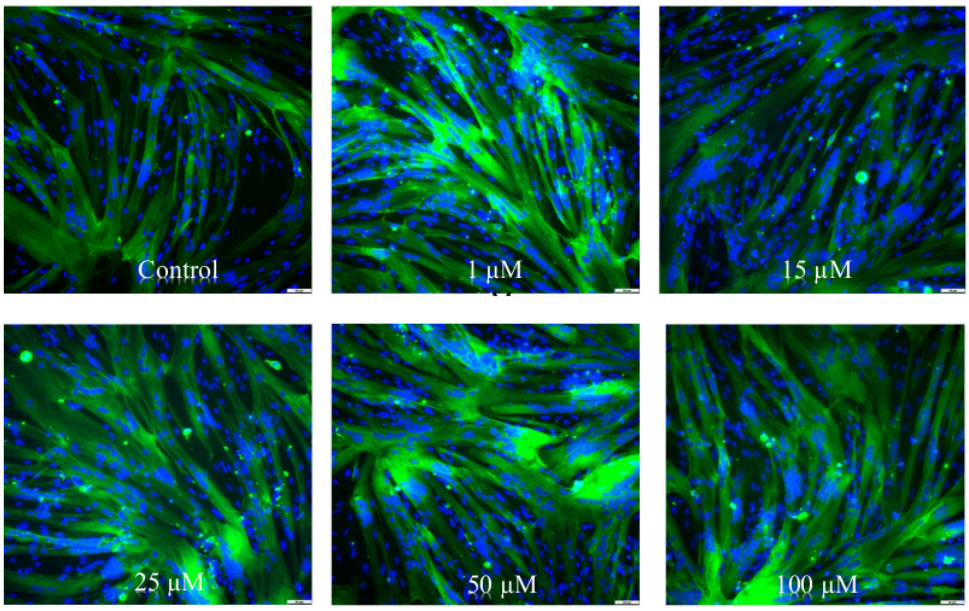

Figure 9. These immunofluorescent images demonstrate the myogenic and morphological changes in myotubes following taurine supplementation at varying concentrations. As taurine concentration increases, there is a notable increase in myotube size and the number of nuclei within the myotubes, which appear more clustered. The most significant growth and development are observed at a concentration of 50 µM, indicating this as the optimal level for promoting myotube formation and maturation.

3.4 Microarray

The microarray analysis assessed the secretion of various growth factors and insulin-like growth factor binding proteins (IGFBPs) in response to different concentrations of taurine in C25 skeletal muscle cells. The table highlights the results for 12 proteins, showing their limits of detection (LOD) and maximum concentrations (MAX) in pg/ml. The data illustrate the protein responses to taurine treatment at concentrations from 1 µM to 100 µM. Notably, significant changes were observed in IGFBP-1, IGFBP-2, IGFBP-3, and IGFBP-6, while the remaining proteins showed minimal or no significant variation.

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Non-Significant Proteins:

- **GDF-15**: Involved in inflammation and apoptosis, but taurine did not significantly impact its levels, suggesting no direct effect on this pathway.
- **GDNF**: Promotes neuron survival and neuromuscular interactions, but showed no significant response, indicating taurine's limited effect on neurotrophic factors.
- **GH**: A key growth and regeneration hormone, but no significant variations were observed, suggesting taurine may not directly regulate GH-related muscle growth.
- **HB-EGF**: Involved in cell growth and wound healing; remained stable with taurine treatment, indicating no major role in taurine's effects on muscle cells.
- **HGF**: Critical for muscle repair, but showed no significant changes, implying other mechanisms may mediate taurine's effects.
- **IGF-1**: Essential for muscle growth, yet showed no significant variation, suggesting taurine's impact may be through IGF regulation rather than IGF-1 itself.
- **Insulin**: Important for muscle metabolism, but unchanged with taurine treatment, indicating its effects may be more related to IGFBPs.

The graphs (Figure 10) illustrate the impact of taurine on the expression and secretion of various IGFBPs, which are key regulators of insulin-like growth factors (IGFs). IGFBP1 levels show a moderate increase at lower taurine concentrations but rise sharply at 100 µM, possibly indicating a stress response or compensatory regulation of IGF activity. IGFBP2 secretion increases modestly at moderate taurine levels (15-50 µM), supporting muscle differentiation and repair, but decreases at 100 µM, suggesting that high taurine concentrations may impair IGF signalling and muscle repair. IGFBP3 levels peak between 25-50 µM, promoting muscle cell survival and regeneration, but plateau at higher concentrations, showing limited additional benefit beyond 50 µM. IGFBP6 secretion rises significantly at 50 µM, supporting muscle differentiation, but spikes at 100 µM, which may indicate a stress response due to excessive taurine(Rosenfeld, 2003) .These findings suggest that moderate taurine levels (15-50 µM) enhance muscle health, while higher concentrations may cause cellular stress.

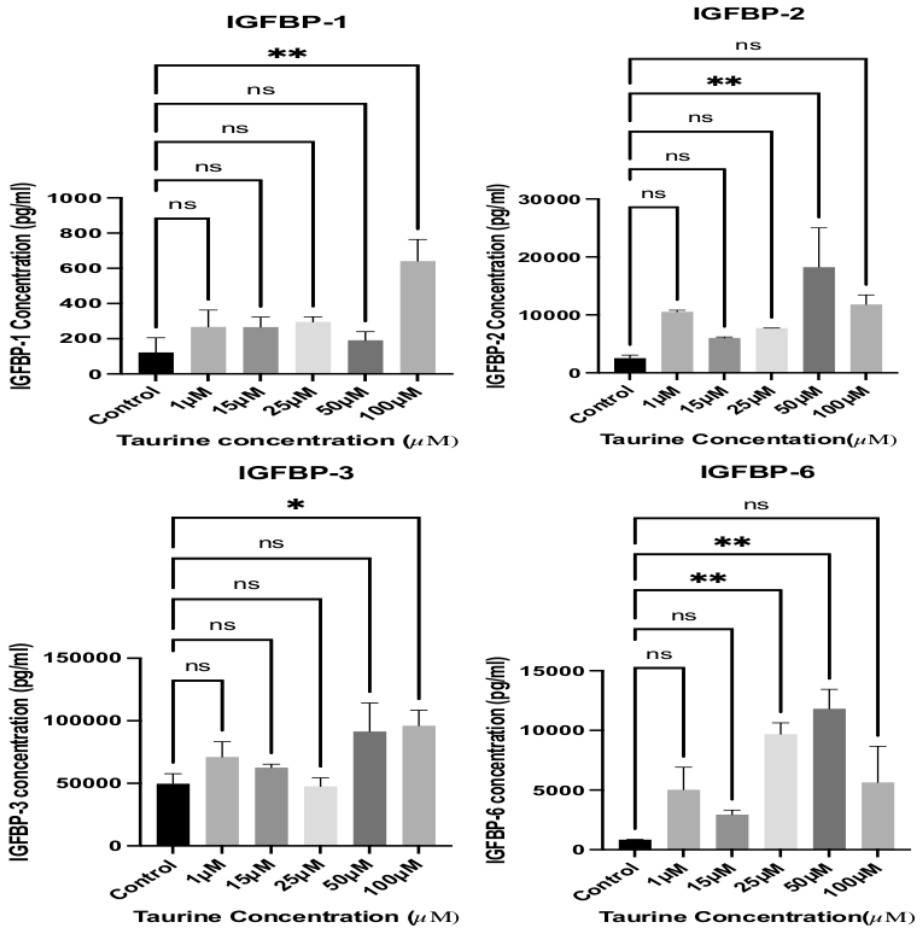

Figure 10. The graphs display the results of a microarray analysis showing the expression of Endogenously secreted growth factors (IGFBP1, IGFBP2, IGFBP3, and IGFBP6) in response to taurine treatment at different concentrations. IGFBP1 and IGFBP6 levels sharply increase at higher taurine concentrations, indicating a potential stress response that could negatively impact muscle differentiation. IGFBP2 shows a gradual increase, supporting its role in muscle differentiation at moderate taurine levels. IGFBP3 peaks at moderate concentrations (25-50 µM), aligning with optimal muscle differentiation outcomes observed in the study.

The results indicate that moderate taurine levels (15-50 µM) support muscle growth, differentiation, and repair by regulating IGFBPs, whereas higher concentrations could cause

cellular stress. This highlights the importance of precise dosing to optimize muscle health benefits.

4.0 DISCUSSION:

This study highlights the significant potential of taurine supplementation in promoting skeletal muscle health, particularly through its positive impact on crucial differentiation parameters such as fusion index, myotube area, and aspect ratio. As outlined in the introduction, skeletal muscles play a fundamental role in enabling movement and regulating vital metabolic processes like glucose absorption and protein storage. These functions are critical for maintaining overall metabolic health and preventing a range of conditions, including sarcopenia, a degenerative loss of muscle mass, and other muscle-wasting diseases. Taurine's ability to enhance muscle cell proliferation, differentiation, and repair makes it a promising candidate for therapeutic interventions to support muscle health. By influencing the structural development of muscle fibres and improving cellular functions, taurine could be particularly beneficial in addressing the muscle degeneration that occurs with ageing or in conditions like Duchenne Muscular Dystrophy (DMD). Furthermore, taurine's role in regulating these processes underscores its potential as a therapeutic supplement, especially in maintaining muscle mass and function in individuals at risk of muscle deterioration. Overall, this research supports the view that taurine could significantly benefit in muscle health preservation and treatment, providing a foundation for further exploration into its use as a clinical intervention.

4.1 Key findings:

The WST-8 cytotoxicity assay revealed that taurine, when administered at low to moderate concentrations (1-50 μM), markedly improvement in cell viability. At these concentrations, skeletal muscle cells displayed notable enhancements in key differentiation markers, including increased fusion, alignment, and expanded myotube area. These improvements are critical indicators of healthy muscle differentiation, suggesting that taurine supports muscle cells' structural and functional maturation. However, as taurine concentrations reached 100 μM, cytotoxic effects began to emerge, evidenced by cellular stress, overcrowding, and reduced cell viability. These adverse effects at higher concentrations align with previous studies suggesting excessive taurine can induce oxidative stress, negating its otherwise beneficial effects. The differentiation assays further confirmed that moderate taurine concentrations (15-50 μM) are optimal for promoting muscle health, with improvements in parameters such as myotube area

and aspect ratio. Among the tested concentrations, 50 µM appeared to offer the most significant benefits for muscle regeneration, with substantial increases in the fusion index and myotube area. These findings suggest that 50 µM may represent the ideal concentration for fostering muscle cell differentiation and growth. This aligns with existing research that emphasises taurine's critical role in supporting muscle cell repair, growth, and overall health. The study underscores taurine's potential as an effective supplement for muscle regeneration, but also highlights the importance of avoiding higher concentrations due to the risk of cytotoxicity.

4.2 IGFBP Expression:

The antibody microarray results reveal that moderate concentrations of taurine (15-50 µM) have a substantial effect on the secretion of insulin-like growth factor binding proteins (IGFBPs), which play a pivotal role in regulating insulin-like growth factor (IGF) signalling pathways crucial for muscle growth, differentiation, and repair Specifically, IGFBP1 and IGFBP3 exhibited increased expression at these moderate taurine levels, suggesting that taurine supports enhanced muscle cell survival and promotes differentiation processes. This aligns with research demonstrating that IGFBP1 and IGFBP3 are key modulators of muscle tissue repair, helping to balance IGF activity and ensure effective muscle regeneration (Rosenfeld et al., 2016). However, when taurine was administered at the higher concentration of 100 µM, there was a pronounced spike in the levels of IGFBP1 and IGFBP6. This significant increase may indicate a cellular stress response, which could negatively impact muscle cell function and interfere with the normal regenerative processes. Elevated IGFBP1 and IGFBP6 at these higher levels suggest that the cells might be attempting to counterbalance the adverse effects of excessive taurine, potentially leading to impaired IGF signalling. This dysregulation could hinder muscle regeneration and trigger cytotoxic effects, emphasizing the importance of maintaining taurine within a moderate range to avoid harmful outcomes and optimize muscle health.

Insulin-like Growth Factor Binding Proteins (IGFBPs) are vital in regulating the availability and activity of insulin-like growth factors (IGFs), which are crucial for muscle growth, differentiation, and repair. These proteins serve as modulators of IGF signalling, either promoting or inhibiting their activity, depending on the cellular context and concentration. In this study, the expression patterns of four significant IGFBPs—IGFBP1, IGFBP2, IGFBP3,

and IGFBP6—were examined after exposing skeletal muscle cells to different concentrations of taurine.

IGFBP-1: It showed a significant increase at the highest taurine concentration (100 μM), indicating a potential cellular stress response. IGFBP1 plays a complex role in muscle physiology, often linked to the negative regulation of IGF signalling. When cells face stress or nutrient scarcity, IGFBP1 levels typically rise, decreasing IGF bioavailability and potentially hindering muscle growth and differentiation (Rajaram et al., 1997). In this study, the elevated IGFBP1 at higher taurine doses suggests that the cells might have been undergoing taurine-induced stress, which could negatively impact muscle regeneration and differentiation at those higher nutrient levels.

IGFBP-2: Its level rose progressively with increasing taurine concentrations, peaking at moderate doses (15-50 μM). This trend suggests that IGFBP2 may be facilitating muscle differentiation and growth. Known to enhance muscle cell proliferation and differentiation, IGFBP2 binds to IGFs, stabilizing and boosting their activity (Jones et al., 2016). The moderate rise in IGFBP2 at these taurine levels corresponds with the improvements seen in muscle differentiation markers, such as the fusion index and myotube area. This indicates that taurine at these concentrations optimizes IGF activity, aiding muscle regeneration and repair.

IGFBP-3: Its expression reached its highest levels at moderate taurine concentrations (25-50 μM), aligning with the most notable muscle differentiation and repair outcomes observed in the study. As one of the most abundant IGFBPs, IGFBP3 supports IGF-mediated muscle growth by extending the half-life of IGFs and amplifying their signalling (Collett-Solberg & Cohen, 1996). The increase in IGFBP3 at these concentrations suggests that taurine, at moderate levels, enhances IGF signalling, fostering muscle differentiation. Moreover, IGFBP3 is associated with protective mechanisms against apoptosis, highlighting its role in supporting muscle cell survival and promoting regeneration when exposed to moderate taurine doses.

IGFBP-6: Its level surged at the highest taurine concentration (100 μM) like IGFBP1. IGFBP6 primarily binds to IGF-II, inhibiting its effects, which are vital for muscle growth and repair. The sharp increase in IGFBP6 at elevated taurine levels indicates a stress-related response, as IGFBP6 is often upregulated under conditions of cellular stress and inflammation (Bach, 2015). This rise suggests the cells were attempting to modulate excessive IGF activity caused by taurine-induced stress, resulting in a suppressive impact on muscle differentiation. These

findings imply that while moderate taurine concentrations support muscle health, higher doses may activate a feedback mechanism that hinders muscle regeneration by increasing IGFBP6 expression.

4.3 Future Directions and Limitations:

While this study highlights the beneficial effects of taurine on skeletal muscle differentiation at moderate concentrations, further research is essential to understand its therapeutic potential. Clinical trials are crucial to validate these in vitro findings and assess taurine's efficacy in treating muscle-related conditions such as Duchenne Muscular Dystrophy (DMD) and sarcopenia associated with ageing. Moreover, future research should focus on evaluating the long-term safety of taurine supplementation, particularly at higher doses, to mitigate the risks of potential cytotoxicity and oxidative stress. Exploring the possibility of combining taurine with other supplements or medications that support muscle health could be valuable. For instance, taurine might work synergistically with antioxidants or calcium regulators, potentially enhancing its benefits in managing exercise-induced muscle fatigue and recovery. However, the study's limitations, particularly its in vitro design, should be considered, as it may not fully reflect the complexity of human muscle tissue. Although the C25 skeletal muscle cell line provides useful insights, in vivo studies are necessary to establish clinical relevance. Additionally, the cytotoxic effects observed at higher taurine concentrations underline the need for precise dosing to minimize adverse effects. Lastly, the limited range of taurine concentrations examined in this study may have overlooked potential outcomes at intermediate levels, indicating that future research should investigate a broader spectrum of taurine doses to capture its full impact.

5.0 CONCLUSION:

This study underscores the promising potential of taurine supplementation in fostering skeletal muscle differentiation, growth, and repair, especially when administered at moderate concentrations (15-50 μM). The results demonstrated that taurine significantly improved key indicators of muscle differentiation, including the fusion index, myotube area, and aspect ratio, all of which are critical to muscle health and development. However, the data also showed that higher taurine concentrations (100 μM) led to cytotoxic effects, highlighting the importance of precise dosing to avoid negative consequences, such as cellular stress or damage. Additionally, the study revealed that taurine plays a regulatory role in the secretion of insulin-like growth factor binding proteins (IGFBPs), which are essential for muscle growth and repair processes. By modulating IGFBP levels, taurine enhances the IGF signalling pathway, supporting muscle regeneration and health. These findings suggest that taurine could be an effective therapeutic agent for muscle-wasting conditions like sarcopenia and Duchenne Muscular Dystrophy (DMD). However, further research is required to fully establish its efficacy and long-term safety. Future studies should include in vivo research and clinical trials to confirm the benefits observed in vitro and to better to assess taurine's therapeutic potential in human populations. Exploring the combination of taurine with other supplements or therapeutic agents, such as antioxidants or calcium regulators, could further enhance its ability to support muscle function and repair. Additionally, addressing the inherent limitations of in vitro models will be crucial in translating these findings into clinical practice, ensuring that taurine can be safely and effectively used to improve muscle health in real-world settings.

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GRADEMARK REPORT

FINAL GRADE

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