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Propofol and Thiopental Disrupt Amino Acid and Carnitine Metabolism in HEK-293 Cells: Insights into Mitochondrial Toxicity

Propofol ve Tiyopental, HEK-293 Hücrelerinde Amino Asit ve Karnitin Metabolizmasını Bozar: Mitokondriyal Toksisiteye Dair Bulgular

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Abstract

Background: Propofol and thiopental are widely used intravenous anesthetics with rapid onset and short duration of action. However, their impact on mitochondrial integrity and cellular metabolism under high-dose exposure remains incompletely characterized.

Materials and Methods: This study employed a metabolomics-based approach, we examined the dose-dependent effects of propofol and thiopental on intracellular amino acid and carnitine metabolism in HEK-293 cells. Cell viability was assessed by MTT assay, and targeted quantification of metabolites was performed via liquid chromatography-mass spectrometry (MS)/MS. All metabolite levels were normalized to total protein content to account for cellular variability.

Results: Both anesthetics caused significant, dose-dependent metabolic alterations. Propofol led to marked depletion of key amino acids (glutamine, alanine, aspartate) and acylcarnitines (C0, C2), indicating compromised mitochondrial β-oxidation and redox homeostasis. Thiopental showed higher cytotoxicity at lower concentrations but induced less disruption in carnitine pathways. Effect size analysis (Cohen's d) confirmed large-to-extreme differences, particularly at 200 µg/mL, underscoring distinct metabolic footprints for each agent.

Conclusions: Propofol and thiopental elicit agent-specific metabolic signatures in renal cells, with implications for mitochondrial dysfunction and anesthetic-induced toxicity. These findings support the utility of targeted metabolomic profiling in guiding safer anesthetic practices, particularly in high-risk or long-duration clinical scenarios.

Keywords: Propofol, Thiopental, Metabolomics, HEK-293 cells, Cellular toxicity

Öz

Amaç: Propofol ve tiyopental, hızlı başlangıç ve kısa etki süresi ile yaygın olarak kullanılan intravenöz anestezik ajanlardır. Ancak bu ajanların yüksek doz maruziyet altında mitokondri bütünlüğü ve hücresel metabolizma üzerindeki etkileri yeterince anlaşılamamıştır.

Materyal ve metod: Bu çalışmada, metabolomik temelli bir yaklaşımla propofol ve tiyopentalin HEK-293 hücrelerinde hücre içi amino asit ve karnitin metabolizması üzerindeki doz bağımlı etkileri incelenmiştir. Hücre canlılığı MTT testi ile değerlendirilmiş; hedeflenmiş metabolit düzeyleri sıvı kromatografisi-kütle spektrometresi (MS)/MS yöntemiyle nicel olarak analiz edilmiştir. Metabolit düzeyleri toplam hücresel protein miktarına normalize edilmiştir.

Bulgular: Her iki anestezik ajan da anlamlı ve doz bağımlı metabolik değişikliklere yol açmıştır. Propofol, temel amino asitler (glutamin, alanin, aspartat) ve asilkarnitinlerde (C0, C2) belirgin azalma oluşturmuş; bu da mitokondriyal β-oksidasyon ve redoks homeostazında bozulmaya işaret etmektedir. Tiyopental daha düşük konsantrasyonlarda daha yüksek sitotoksisite göstermiş ancak karnitin metabolizmasında daha az bozulmaya neden olmuştur. Etki büyüklüğü analizi (Cohen's d), özellikle 200 µg/mL dozda olmak üzere, iki ajan arasında büyükten aşırıya değişen farklar olduğunu doğrulamıştır.

Sonuç: Propofol ve tiyopental, böbrek hücrelerinde ajanlara özgü metabolik izler oluşturmakta olup bu farklılıklar mitokondriyal disfonksiyon ve anestezi kaynaklı toksisite açısından klinik önem taşımaktadır. Elde edilen bulgular, hedeflenmiş metabolomik profil analizlerinin, özellikle yüksek riskli veya uzun süreli anestezik maruziyet gerektiren durumlarda daha güvenli anestezi uygulamalarını yönlendirmede değerli bir araç olabileceğini göstermektedir.

Anahtar Kelimeler: Propofol, Tiyopental, Metabolomik, HEK-293 hücreleri, Hücresel toksisite

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Introduction

Propofol and thiopental are cornerstone agents of modern intravenous anesthesia, valued for their rapid onset and short duration of action of action (1-3). Despite their widespread clinical use, emerging evidence indicates that these anesthetics exert significant biochemical effects on cellular metabolism and mitochondrial function. Understanding their distinct cellular impacts is essential for optimizing anesthetic selection in vulnerable populations and minimizing the risk of metabolic toxicity.

Propofol is a short-acting intravenous anesthetic agent widely utilized for both the induction and maintenance of general anesthesia (1,4). Due to its rapid onset, brief duration of action, and favorable recovery profile, it remains one of the most commonly preferred agents in contemporary anesthetic practice. Its primary mechanism of action involves potentiation of inhibitory neurotransmission via gamma-aminobutyric acid type A (GABA-A) receptors, leading to central nervous system depression (2). In addition to its anesthetic effects, Propofol exhibits antiemetic, anticonvulsant, and antioxidant properties, further contributing to its clinical versatility (5).

Thiopental (sodium thiopental) was the most widely used intravenous anesthetic agent prior to the introduction of Propofol and continues to hold clinical relevance in specific settings (6). As a member of the barbiturate class, Thiopental enhances inhibitory neurotransmission by activating GABA-A receptors, resulting in central nervous system depression. Due to its rapid induction properties, anticonvulsant activity, and neuroprotective effects, it remains a preferred agent in the management of status epilepticus and in patients with elevated intracranial pressure (7).

Both agents exhibit pharmacokinetic profiles characterized by rapid onset, short duration of action of action, and high clearance rates, rendering them well-suited for the induction of short-term anesthesia (8). Although Propofol and Thiopental are generally considered safe at therapeutic doses, their administration at high doses or for prolonged durations may lead to various toxic effects (9). Such usage can result in drug accumulation in plasma and tissues, increasing systemic concentrations and elevating the risk of dose-related toxicity (3,4).

One of the most frequently reported adverse effects associated with propofol is injection site pain, particularly when administered into smaller veins. In addition, it may cause hypotension, bradycardia, respiratory depression, and localized irritation at the injection site (10). Among the most severe complications is Propofol Infusion Syndrome (PRIS), a rare but life-threatening condition characterized by refractory bradycardia that may progress to asystole, particularly during prolonged high-dose infusions (11). In contrast, thiopental carries a risk of severe anaphylaxis with an estimated incidence of 1 in 20,000 administrations. Extravasation may lead to tissue necrosis, and extended infusion has been associated with immunosuppressive effects (12).

The cellular metabolic effects of anesthetic agents particularly their influence on amino acid and carnitine pathways have garnered increasing scientific attention in recent years (13). These agents can disrupt mitochondrial function and alter intracellular energy Dynamics (14). Carnitine plays a pivotal role in mitochondrial metabolism, primarily by facilitating the transport of long-chain fatty acids into mitochondria for β-oxidation (15). Shifts in carnitine derivative profiles may therefore serve as sensitive markers of mitochondrial dysfunction.

HEK-293 cells, derived from human embryonic kidney tissue, are widely utilized as an in vitro model for preliminary toxicity screening due to their consistent growth characteristics and high transfection efficiency (16). Their robust and reproducible nature makes them highly suitable for experimental manipulation, and they serve as a relevant platform for evaluating potential renal toxicity of pharmaceutical compounds (17). While HEK-293 cells offer a useful screening platform, they do not recapitulate all tissue-specific responses to anesthetic exposure.

Metabolomics provides a systems-level perspective on biochemical alterations and has emerged as a powerful tool in toxicology research (18). Profiling of amino acids and carnitine metabolites is particularly valuable, as these biomolecules are key components of cellular metabolic pathways and serve as precise indicators of physiological stress or dysfunction (19). By enabling the identification of perturbations in metabolic networks, metabolomic approaches offer unique insights into the mechanistic underpinnings of drug-induced toxicity (20).

This study investigates the comparative effects of high-dose propofol and thiopental on amino acid and carnitine metabolism in HEK-293 cells. The aim was to evaluate the dose-dependent toxicological responses of these intravenous anesthetics and to explore the underlying mechanisms of their metabolic impact using a metabolomics-based approach. By analyzing drug-induced alterations in intracellular amino acil and carnitine profiles, we sought to elucidate potential disruptions in mitochondrial function and cellular energy homeostasis triggered by propofol and thiopental exposure.

Material and Methods

Cell Line and Culture Conditions

In this study, we used human embryonic kidney (HEK-293) cells, an immortalized epithelial cell line obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA; ATCC number: CRL-1573) (21). All experimental procedures adhered to the Checklist for Reporting In Vitro Studies guidelines to ensure methodological transparency and reproducibility (22).

Cells were cultured in Dulbecco's Modified Eagle Medium (DMEM, Gibco, USA) supplemented with 10% fetal bovine serum, 1% penicillin-streptomycin (P/S), and 1% L-glutamine, and maintained in a humidified incubator at 37°C with 5% CO_2. Subculturing was performed at a ratio of 1:2 or 1:3 using a 0.25% trypsin EDTA solution, following ATCC-recommended procedures.

For long-term storage, surplus cells were suspended in a cryopreservation medium consisting of 95% complete growth medium and 5% dimethyl sulfoxide. The cryovials were initially stored at -80°C and subsequently transferred to liquid nitrogen for long-term preservation.

Drug Exposure Protocol

Drug Treatment and Cytotoxicity Evaluation (MTT Assay)

Twenty-four hours after seeding HEK-293 cells in sterile 96-well plates at a density of $1x10^4$ cells/well, the medium was replaced with fresh complete medium. Ten concentrations of Propofol and Thiopental (0, 2.5, 5, 10, 25, 50, 100, 200, 250, and 400-500 µg/mL) were applied in triplicate. Untreated cells served as controls. Following drug administration, the cells were incubated for an additional 24 hours at 37°C in a 5% CO_2 environment.

After incubation, the medium was aspirated, and cytotoxicity was evaluated using the MTT assay. Absorbance was measured at 570 nm (reference: 690 nm) with a microplate reader (Thermo Multiskan Go, USA). Dose-response curves were constructed, and IC_{50} values were calculated. Based on these values, 100 µg/mL and 200 µg/mL were selected as the working concentrations for further analyses..

Free Amino Acid Profiling by LC-MS/MS

Free amino acid levels were quantified using a Shimadzu LCMS-8045 system according to a modified version of the method by Celik et al. (23), along with a commercial derivatization kit (Trimaris).

Samples were prepared in 0.1 M HCl and labeled with $^{13}C/^{15}N$-labeled internal standards. Following mixing with a basic organic buffer in propanol to facilitate derivatization and protein precipitation, a chloroform-isooctane mixture containing 5% alkyl chloroformate was added. After 3 minutes of incubation, the upper organic phase was isolated by centrifugation, and 1 µL of the derivatized sample was injected into the LC-MS/MS system.

Chromatographic separation was carried out on a reversed-phase C18 column (Trimaris Amino Acid column, 250 mm x 2 mm, 3 µm). Mobile phase A consisted of water:methanol:1 M ammonium formate (85:14:1), and mobile phase B was pure methanol. Detection was performed under ESI+ conditions with MRM acquisition.

Cell Homogenization and Carnitine Profiling

Post-treatment, cells were detached by trypsinization and centrifuged at 1,000 rpm for 5 minutes. The pellet was resuspended in cold lysis buffer and homogenized using a TissueLyser system. The lysate was then centrifuged, and the supernatant collected.

A 5 µL aliquot was spotted onto Guthrie cards and air-dried overnight at room temperature. The dried samples were cut and transferred into microtubes. Carnitine profiling was conducted following established protocols by La Marca et al. (24), Azzari et al. (25), and Saglik et al. (26). Each sample was treated with 200 µL of internal standard solution and dried under nitrogen for 30 minutes. Then, 60 µL of butanolic-HCl derivatization reagent was added, incubated at 60°C for 30 minutes, and dried again. Samples were reconstituted in 100 µL of mobile phase (acetonitrile:methanol:formic acid) and analyzed by LC-MS/MS using an LCMS-8040 system (Shimadzu Corporation, Kyoto, Japan) in ESI+ mode with MRM detection

Statistical Analysis

All statistical analyses were conducted using IBM SPSS Statistics version 24.0 (IBM Corp., Armonk, NY, USA). Normality was assessed via the Kolmogorov-Smirnov and Shapiro-Wilk tests. Continuous data were presented as mean ± SD. One-way ANOVA was used to evaluate differences in amino acid and carnitine levels across treatment groups (0, 100, and 200 µg). Tukey's post hoc test was applied where significance was found. Direct comparisons between Propofol and Thiopental at equivalent doses were performed using independent-samples Student's t-tests. A two-tailed $p<0.05$ was considered statistically significant. Additionally, Cohen's d values were calculated to quantify the magnitude of observed effects.

Results

HEK-293 Cell Viability Following Propofol and Thiopental Exposure

As part of a previously published investigation evaluating the cytotoxic, oxidative, and apoptotic effects of Propofol and Thiopental in HEK-293 cells, cell viability was assessed using the MTT assay (4). In the current study, those viability data were directly adopted to avoid redundancy, as the experimental conditions including cell line, culture parameters, drug concentrations, and exposure durations were kept identical.

The previously reported IC_{50} values were 206.59 µg/mL for Propofol and 109.68 µg/mL for Thiopental, indicating a stronger cytotoxic potential for Thiopental. Significant reductions in viability compared to control (0 µg/mL) were observed at ≥25 µg/mL for Thiopental and ≥50 µg/mL for Propofol ($p<0.001$). Furthermore, head-to-head comparisons at corresponding doses revealed that Propofol was significantly less cytotoxic than Thiopental at 200 µg/mL, 250 µg/mL, and 500 µg/mL ($p<0.001$). These data provide the foundational rationale for selecting 100

µg/mL and 200 µg/mL as experimental doses in the present metabolomic profiling of amino acid and carnitine pathways.

Comparative Metabolomic Impact of Propofol and Thiopental on HEK-293 Cells

Table 1 summarizes the changes in intracellular amino acid concentrations in HEK-293 cells following exposure to two widely used intravenous anesthetics, Propofol and Thiopental, at concentrations of 100 µg and 200 µg. Untreated cells (0 µg) served as the control group. For each amino acid, mean concentrations and standard deviations (µM) are reported under all experimental conditions. Statistical significance was determined using one-way ANOVA followed by Tukey's post-hoc test, and inter-group comparisons were conducted at matched doses.

Both anesthetics elicited dose-dependent depletion of multiple amino acids involved in key cellular processes such as energy production, redox regulation, and neurotransmitter synthesis. Significant reductions were observed in Alanine, Arginine, Glutamine, and Aspartic Acid levels, with Propofol generally exerting a more pronounced suppressive effect, particularly at the 200 µg concentration. These metabolic alterations may reflect mitochondrial stress, enhanced amino acid catabolism, or shifts in biosynthetic demand induced by anesthetic exposure.

The p-values presented in the final columns of the table highlight statistically significant differences between Propofol and Thiopental at equivalent doses, revealing agent-specific metabolic signatures. These findings underscore the value of metabolomic profiling in delineating the cellular effects of anesthetic agents and provide insights into their differential toxicity, recovery potential, and suitability for both experimental and clinical applications.

Dose-Dependent Impairment of Carnitine-Mediated β-Oxidation by Intravenous Anesthetics in Renal Cells

Table 2 presents the quantitative alterations in intracellular free carnitine (C0) and a broad panel of short-, medium-, and long-chain acylcarnitines in HEK-293 cells following treatment with Propofol and Thiopental at clinically relevant concentrations (100 µg and 200 µg). Control cells (0 µg) were used as a baseline. Values are expressed as mean ± standard deviation (µM). Statistical comparisons were performed using one-way ANOVA with Tukey's post-hoc test. Significant differences are denoted as follows: *p<0.05, **p<0.01 compared to the control group; &p <0.05, &&p <0.01 for comparisons between Propofol and Thiopental at equivalent doses.

Exposure to both anesthetic agents resulted in significant, dose-dependent disruptions in carnitine homeostasis an essential regulatory axis for mitochondrial fatty acid uptake, β-oxidation, and ATP production. Propofol, in particular, elicited a pronounced depletion of free carnitine (C0) and multiple short- and medium-chain acylcarnitines at both concentrations tested, with the most severe reductions observed at 200 µg. This pattern suggests an anesthetic-induced impairment of the carnitine shuttle system, potentially through inhibition of carnitine transporters (e.g., OCTN2) or secondary to mitochondrial membrane dysfunction, leading to reduced substrate availability for oxidative phosphorylation. The loss of these critical intermediates may reflect increased demand for fatty acid metabolism under stress or represent a direct inhibitory effect of Propofol on mitochondrial β-oxidation pathways.

In contrast, Thiopental exposure was associated with a more selective or attenuated modulation of carnitine species, indicating a comparatively milder impact on mitochondrial bioenergetic processes. While some alterations were noted, especially at higher doses, the overall profile suggests preserved carnitine-mediated fatty acid flux and reduced mitochondrial vulnerability compared to Propofol-treated cells. This differential response may stem from distinct molecular mechanisms of action between the two agents, including their lipid solubility, membrane interactions, or downstream effects on oxidative metabolism.

The final three columns of the table provide direct statistical comparisons between Propofol and Thiopental at matched doses (100 µg and 200 µg), revealing divergent metabolic fingerprints. These inter-agent differences emphasize that anesthetic agents, though clinically interchangeable in certain contexts, can elicit profoundly distinct effects at the cellular level. Collectively, these findings underscore the utility of targeted metabolomic profiling for uncovering subtle but critical variations in drug-induced mitochondrial dysfunction. Furthermore, they offer mechanistic insights into how intravenous anesthetics may contribute to postoperative fatigue, metabolic instability, or organ-specific toxicity particularly in vulnerable populations such as neonates, critically ill patients, or those with pre-existing mitochondrial disorders.

Effect Size Analysis Reveals Distinct Metabolic Footprints of Propofol and Thiopental in HEK-293 Cells

To complement statistical significance testing, we conducted a quantitative assessment of metabolic alterations using Cohen's d effect size analysis (Figure 1). This approach enabled the comparison of magnitude and directionality in metabolite changes between Propofol and Thiopental exposure in HEK-293 cells across two dose levels (100 µg and 200 µg).

Table 1. Anesthetic-induced remodeling of amino acid metabolism: a comparative analysis of propofol and thiopental in HEK-293 cells

	Control		Propofol					Thiopental					Propofol vs Thiopental	
Dose	**0 µg**		**100 µg**		**200 µg**			**100 µg**		**200 µg**			**100 µg**	**200 µg**
	Mean	**Std. deviation**	**Mean**		**Std. deviation**		**p-value**	**Mean**		**Std. deviation**		**p-value**	**p-value**	**p-value**
Alanine	15.548	1.022	8.871	0.583	2.794	0.184	<0.001**	7.619	0.501	2.932	0.193	<0.001**	<0.001&&	<0.001&&
Arginine	22.255	1.558	12.658	0.886	5.506	0.385	<0.001**	12.741	0.892	5.959	0.417	<0.001**	<0.001&&	<0.001&&
Asparagine	4.426	0.330	4.825	0.360	1.697	0.127	<0.001**	4.781	0.357	1.715	0.128	<0.001**	<0.001&&	<0.001&&
Aspartic_Acid	18.320	1.456	13.466	1.070	3.990	0.317	<0.001**	14.720	1.170	5.628	0.447	<0.001**	<0.001&&	<0.001&&
Citrulline	1.732	0.147	0.600	0.051	0.117	0.010	<0.001**	0.955	0.081	0.357	0.030	<0.001**	<0.001&&	<0.001&&
Glutamine	662.443	59.542	253.411	22.777		23.732	<0.001**	399.453	35.904	201.854	18.143	<0.001**	0.606	0.001&
Glutamic_Acid	40.614	3.873	23.378	2.230	8.152	0.777	<0.001**	20.166	1.923	8.515	0.812	<0.001**	<0.001&&	0.001&
Glycine	26.829	2.710	23.614	2.385	7.906	0.799	<0.001**	23.521	2.376	8.269	0.835	<0.001**	<0.001&&	<0.001&&
Histidine	3.093	0.330	1.486	0.159	0.810	0.086	<0.001**	1.714	0.183	0.611	0.065	<0.001**	0.003&	0.001&
Leucine	19.673	2.217	8.008	0.902	4.348	0.490	<0.001**	8.798	0.991	3.899	0.439	<0.001**	0.003&	0.001&
Isoleucine	9.716	1.153	3.712	0.441	2.263	0.269	<0.001**	4.004	0.475	1.832	0.217	<0.001**	0.008&	0.002&
Alloisoleucine	0.176	0.022	0.073	0.009	0.040	0.005	<0.001**	0.077	0.010	0.034	0.004	<0.001**	0.005&	0.002&
Lysine	17.190	2.253	9.044	1.185	3.450	0.452	<0.001**	9.027	1.183	3.410	0.447	<0.001**	0.002&	0.002&
Methionine	3.072	0.994	1.654	0.535	0.624	0.202	0.011*	1.566	0.506	0.672	0.217	0.012*	0.036&	0.048&
Ornithine	3.150	1.050	1.378	0.459	0.632	0.211	0.010*	1.717	0.572	0.663	0.221	0.014*	0.063	0.041&
Phenylalanine	2.772	0.951	1.407	0.483	0.680	0.233	0.018*	1.398	0.480	0.582	0.200	0.014*	0.079	0.053
Proline	16.105	5.691	8.704	3.076	2.699	0.954	0.014*	8.300	2.933	3.010	1.064	0.015*	0.032&	0.043&
Serine	4.410	1.605	2.771	1.008	1.221	0.444	0.037*	2.623	0.954	1.213	0.441	0.034*	0.071	0.081
Threonine	9.580	3.589	5.717	2.142	2.536	0.950	0.036*	6.191	2.319	2.823	1.057	0.047*	0.078	0.084
Tryptophan	1.924	0.264	1.045	0.144	0.505	0.069	<0.001**	1.122	0.154	0.463	0.064	<0.001**	0.004	0.002&
Tyrosine	7.505	1.079	3.997	0.575	1.930	0.277	<0.001**	3.979	0.572	1.691	0.243	<0.001**	0.005&	0.003&
Valine	8.428	1.613	3.884	0.743	1.815	0.347	0.001*	4.284	0.820	1.816	0.348	0.001**	0.012&	0.009&
Alphaaminoadipic_Acid	0.037	0.007	0.017	0.003	0.021	0.004	0.009*	0.249	0.050	0.010	0.002	<0.001**	0.346	0.001&
Alphaaminopimelic_Acid	0.468	0.083	0.485	0.086	0.486	0.086	0.959	0.474	0.084	0.456	0.081	0.965	0.991	0.805
Anserine	5.989	1.104	5.568	1.026	5.506	1.015	0.833	6.116	1.127	5.859	1.080	0.960	0.944	0.789
Betaaminoisobutyric_Acid	0.214	0.085	0.003	0.001	0.048	0.019	0.005*	0.094	0.037	0.037	0.015	0.019*	0.015&	0.070
Gammaminobutyric_Acid	0.374	0.088	0.006	0.001	0.004	0.001	<0.001**	0.008	0.002	0.004	0.001	<0.001**	0.164	0.031&
Beta_Alanine	0.457	0.112	0.115	0.028	0.101	0.025	0.001*	0.002	0.001	0.093	0.023	<0.001**	0.541	0.002&
Sarcosine	0.539	0.136	0.194	0.049	0.062	0.016	0.001*	0.268	0.068	0.103	0.026	0.003*	0.011&	0.017&
Cystathionine	1.311	0.342	0.577	0.151	0.189	0.049	0.002*	0.616	0.161	0.233	0.061	0.003*	0.013&	0.018&
Thiaproline	0.566	0.152	0.294	0.079	0.059	0.016	0.002*	0.234	0.063	0.080	0.021	0.002*	0.007&	0.016&
Methylhistidine_1	0.369	0.102	0.142	0.039	0.111	0.031	0.006*	0.158	0.044	0.064	0.018	0.003*	0.343	0.027&
Methylhistidine_3	0.028	0.008	0.015	0.004	0.007	0.002	0.008*	0.011	0.003	0.002	0.001	0.002*	0.045&	0.010&
Hydroxylysine	0.023	0.007	0.001	0.000	0.004	0.001	0.001*	0.001	0.000	0.004	0.001	0.001*	0.010&	0.010&

Table 1. Continued														
Hydroxyproline	0.152	0.046	0.004	0.001	0.087	0.027	0.003*	0.238	0.073	0.164	0.050	0.220	0.006&	0.217
Cystine	3.702	1.162	1.573	0.494	0.830	0.261	0.008*	1.691	0.531	0.748	0.235	0.008*	0.083	0.048&
Histamine	0.007	0.001	0.01	0.00	0.009	0.001	0.009*	0.007	0.001	0.002	0.000	0.001*	1.000	0.002&
Etanolamine	0.623	0.128	0.19	0.04	0.148	0.030	0.054	0.214	0.044	0.104	0.021	<0.001**	0.007&	0.018&
Phosphoetanolamine	21.087	4.500	7.802	1.665	27.532	5.875	0.004	2.792	0.596	22.086	4.713	0.001*	0.005&	0.002&
Oh_Trp_5	0.027	0.006	0.10	0.02	0.369	0.081	0.696	0.135	0.030	0.431	0.095	<0.001**	0.031&	0.007&
Taurine	4.739	1.083	23.10	5.28	3.062	0.700	4.018	5.233	1.196	5.038	1.152	0.871	0.225	0.849

Values are expressed as mean ± standard deviation (µM). Statistically significant differences were assessed using one-way ANOVA followed by Tukey's post-hoc test. Asterisks (*) indicate comparisons versus the control group (0 µg), with *p<0.05 and **p<0.01. Ampersands (&) denote comparisons between Propofol and Thiopental at equivalent doses (100 µg and 200 µg), with &p<0.05 and &&p<0.01.

Table 2. Comparative effects of propofol and thiopental on intracellular carnitine and acylcarnitine profiles in HEK-293 cells

	Control		**Propofol**					**Thiopental**					**Propofol vs Thiopental**	
	0 µg		**100 µg**		**200 µg**			**100 µg**		**200 µg**			**100 µg**	**200 µg**
	Mean	**Std. deviation**	**Mean**	**Std. deviation**	**Mean**	**Std. deviation**	**p-value**	**Mean**	**Std. deviation**	**Mean**	**Std. deviation**	**p-value**	**p-value**	**p-value**
C0	0.5167	0.0058	0.2633	0.0153	0.8700	0.0346	0.026*	0.6267	0.0351	0.1867	0.0153	0.027*	<0.001&&	0.046&
C2	1.7700	0.0200	1.0367	0.0306	0.8867	0.0153	<0.001**	1.9100	0.1100	0.6867	0.0379	<0.001**	<0.001&&	0.001&
C3	0.2300	0.0100	0.1900	0.0100	0.1600	0.0100	0.027*	0.2033	0.0321	0.1400	0.0173	0.054	0.530	0.178
C4	0.0500	0.0100	0.0400	0.0000	0.0333	0.0058	0.071	0.0767	0.0462	0.0633	0.0404	0.538	0.034&	0.099
C4DC	0.0000	0.0000	0.0000	0.0000	0.0033	0.0058	0.368	0.0033	0.0058	0.0000	0.0000	0.368	0.317	0.317
C5	0.0567	0.0058	0.0400	0.0000	0.0300	0.0000	0.020*	0.0533	0.0231	0.0367	0.0115	0.185	0.317	0.317
C5_1	0.0067	0.0058	0.0033	0.0058	0.0033	0.0058	0.670	0.0133	0.0058	0.0033	0.0058	0.179	0.099	1.000
C5OH	0.0567	0.0058	0.0533	0.0058	0.0467	0.0058	0.179	0.0600	0.0173	0.0500	0.0100	0.612	0.796	0.637
C5DC	0.0033	0.0058	0.0100	0.0000	0.0067	0.0058	0.264	0.0100	0.0000	0.0033	0.0058	0.202	1.000	0.456
C6	0.0067	0.0058	0.0033	0.0058	0.0067	0.0058	0.670	0.0167	0.0115	0.0100	0.0100	0.464	0.099	0.637
C6DC	0.0067	0.0058	0.0067	0.0058	0.0067	0.0058	1.000	0.0133	0.0058	0.0067	0.0058	0.304	0.197	1.000
C8	0.0000	0.0000	0.0033	0.0058	0.0000	0.0000	0.368	0.0067	0.0058	0.0067	0.0058	0.202	0.456	0.114
C8_1	0.0067	0.0058	0.0033	0.0058	0.0067	0.0058	0.670	0.0100	0.0000	0.0067	0.0058	0.565	0.114	1.000
C8DC	0.0067	0.0058	0.0100	0.0000	0.0067	0.0058	0.565	0.0100	0.0100	0.0067	0.0115	0.848	1.000	0.814
C10	0.0033	0.0058	0.0000	0.0000	0.0033	0.0058	0.565	0.0100	0.0100	0.0100	0.0100	0.544	0.121	0.346
C10_1	0.0067	0.0058	0.0100	0.0000	0.0100	0.0000	0.368	0.0133	0.0153	0.0167	0.0115	0.513	1.000	0.317
C10DC	0.0300	0.0000	0.0267	0.0058	0.0267	0.0058	0.565	0.0333	0.0058	0.0233	0.0058	0.110	0.197	0.456
C12	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.000	0.0033	0.0058	0.0033	0.0058	0.565	0.317	0.317
C14	0.0033	0.0058	0.0100	0.0000	0.0067	0.0058	0.264	0.0167	0.0115	0.0133	0.0058	0.136	0.317	0.197

Table 2. Continued

C14_1	0.0000	0.0000	0.0000	0.0000	0.0000	1.000	0.0067	0.0058	0.0067	0.0058	0.202	0.114	0.114
C14_2	0.0067	0.0058	0.0067	0.0058	0.0100	0.0000	0.565	0.0133	0.0058	0.0133	0.0058	0.304	0.197	0.317
C16	0.0100	0.0000	0.0033	0.0058	0.0200	0.0000	0.029	0.0233	0.0058	0.0133	0.0058	0.056	0.043[&]	0.114
C16_1	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.000	0.0067	0.0058	0.0033	0.0058	0.264	0.114	0.317
C18	0.0100	0.0000	0.0100	0.0000	0.0100	0.0000	1.000	0.0133	0.0058	0.0100	0.0100	0.740	0.317	1.000
C18_1	0.0067	0.0058	0.0067	0.0058	0.0067	0.0058	1.000	0.0067	0.0115	0.0067	0.0058	0.961	0.814	1.000
C18_2	0.0000	0.0000	0.0033	0.0058	0.0000	0.0000	0.368	0.0033	0.0058	0.0033	0.0058	0.565	1.000	0.317
C18_1_OH	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	1.000	0.0033	0.0058	0.0033	0.0058	0.565	0.317	0.317

Values are expressed as mean ± standard deviation (µM). Statistically significant differences were determined using one-way ANOVA followed by Tukey's post-hoc test. Asterisks indicate comparisons versus the control group (0 µg), where *p<0.05 and **p<0.01. Ampersand symbols denote differences between Propofol and Thiopental at equivalent doses (100 µg or 200 µg), with & p<0.05 and && p<0.01.

At the 100 µg dose, Propofol induced notably large suppressive effects on several amino acids. Alanine exhibited a substantial decrease (d=-2.30), while glutamine showed an exceptionally large negative effect size (d=-4.86) relative to Thiopental, indicating marked disruption of amino acid homeostasis. In contrast, arginine remained relatively stable (d=-0.09), suggesting limited divergence between agents for this metabolite at this dose.

In the carnitine metabolism pathway, Propofol produced dramatic depletions in both free carnitine (C0) and acetylcarnitine (C2) with effect sizes of d=-13.42 and d=-10.82, respectively. These values far exceeded conventional thresholds for large effects (d>0.8), indicating a profound impact on mitochondrial β-oxidation and substrate transport.

At the 200 µg dose, the effect size patterns demonstrated both dose dependency and directional reversals for select metabolites. For instance, Propofol led to a notable increase in glutamine (d=2.94) and reversed its effect on C0 (d=25.54) relative to Thiopental. Acetylcarnitine (C2) also showed a strong differential elevation (d=6.92), consistent with an altered mitochondrial response. Meanwhile, alanine and arginine remained negatively affected (d=-0.73 and d=-1.13, respectively), suggesting ongoing suppressive effects.

These findings collectively indicate that Propofol and Thiopental exert distinct and dose-dependent metabolic pressures on both cytoplasmic and mitochondrial pathways. The magnitude and variability of effect sizes point toward a more dynamic and potentially disruptive influence of Propofol on cellular energy metabolism and stress adaptation mechanisms.

Discussion

In this study, we investigated the comparative effects of two widely used intravenous anesthetics, Propofol and Thiopental, on intracellular amino acid and carnitine metabolism in HEK-293 cells using a metabolomics-based approach. Our findings revealed distinct dose-dependent alterations in key metabolic pathways involved in mitochondrial function and energy homeostasis. Notably, Propofol induced a more pronounced suppression of several amino acids and carnitine derivatives compared to Thiopental, suggesting differential modulation of bioenergetic and redox processes. These results build upon previous evidence of anesthetic-induced mitochondrial stress and provide novel insights into the agent-specific metabolic signatures that may underlie differences in cytotoxicity and cellular adaptation. The use of HEK-293 cells as a renal epithelial model further underscores the relevance of these findings in the context of nephrotoxicity and systemic anesthetic safety.

In our study, both Propofol and Thiopental induced significant alterations in amino acid metabolism in HEK-293 cells. Notably,

both anesthetics led to marked reductions in essential amino acids such as valine, leucine, isoleucine, and phenylalanine. These findings suggest potential interference with protein synthesis and cellular energy metabolism, processes highly dependent on amino acid availability.(4) Recent metabolomic studies by Garmon et al. demonstrated that anesthetic agents undergo complex hepatic biotransformation via multiple cytochrome P450 isoforms, potentially disrupting amino acid turnover and related metabolic pathways (27). Furthermore, post-Propofol exposure changes in neurotransmitter-associated amino acids particularly glutamate and GABA may reflect early mechanistic events contributing to its known neurotoxicity profile (2).

Alterations in carnitine derivatives are critically important for understanding mitochondrial function and cellular energy metabolism. In our study, significant changes were observed in carnitine profiles following exposure to both Propofol and Thiopental. It has been reported that Propofol elevates malonyl-CoA levels, thereby inhibiting carnitine palmitoyltransferase I (CPT I) activity and impairing the mitochondrial uptake of long-chain fatty acids (28). A recent study by Tan et al. demonstrated that anesthetic agents can influence various aspects of mitochondrial physiology, including morphology, dynamics, energy metabolism, and protein expression profiles (14). Notably, the pathophysiological mechanism of PRIS is thought to be closely linked to mitochondrial dysfunction, reinforcing the relevance of carnitine metabolism as a sensitive indicator of anesthetic-induced bioenergetic impairment (11).

The observed metabolic alterations are strongly implicated in underlying cellular toxicity mechanisms. In a recent study by Pehlivan et al., Propofol and Thiopental were shown to exert significant cytotoxic effects on HEK-293 cells at supratherapeutic concentrations, primarily mediated through increased intracellular reactive oxygen species (ROS) production and apoptosis induction (4). Similarly, Zhang et al. (29) demonstrated that anesthetic agents inhibit mitochondrial respiration, reduce ATP synthesis, and impair mitochondrial membrane potential, thereby contributing to cellular toxicity (29). In addition, a study examining the toxic effects of local anesthetics on cardiac cells reported that these agents disrupt intracellular calcium homeostasis and induce mitochondrial dysfunction, ultimately leading to cardiotoxicity (30).

The translational relevance of our in vitro findings is particularly significant in the context of high-dose anesthetic exposure. Wang et al. (9) reported that PRIS is typically associated with prolonged administration (>48 hours) and high cumulative doses (>4 mg/kg/hour). In clinical practice, plasma concentrations of Propofol and Thiopental typically reach approximately 11 µg/mL and 41.6 µg/mL, respectively; however, prolonged or high-dose infusions particularly of Propofol can exceed these levels and precipitate severe toxic syndromes. For instance, PRIS, observed in 2.9% of cases with a mortality rate of 36.8%, has been associated with cumulative doses exceeding 276.5 mg/kg and infusion durations surpassing 147 hours, leading to complications such as metabolic acidosis, cardiac dysfunction, hypertriglyceridemia, and rhabdomyolysis (4,31). In contrast, our study identified IC_{50} values of 206.59 µg/mL for Propofol and 109.68 µg/mL for Thiopental in HEK-293 cells, suggesting that overt cytotoxicity emerges primarily at concentrations exceeding therapeutic levels.

Altered amino acid and carnitine profiles may impact several metabolic pathways, with potential implications for cell survival. In particular, Propofol and Thiopental were observed to influence critical energy-related pathways such as mitochondrial β-oxidation, the tricarboxylic acid cycle, and oxidative phosphorylation (32). A study by Borzage et al. (2) further reinforced that mitochondrial impairment can occur regardless of species, exposure method, anesthetic type, or timing, underscoring the vulnerability of mitochondrial integrity to anesthetic challenge. These metabolic disturbances may compromise cellular bioenergetics and enhance oxidative stress, thereby reducing cell viability.

Comparing the toxicity profiles of Propofol and Thiopental provides valuable insight into their relative safety. Our findings demonstrate that the IC_{50} value of Thiopental (109.68 µg/mL) was considerably lower than that of Propofol (206.59 µg/mL), indicating a higher cytotoxic potential for Thiopental under identical conditions (4). Moreover, Propofol exhibited a more favorable cytotoxicity profile across several tested doses. In support of this, Arslan et al. (6) reported that although both anesthetics may induce hypotension through nitric oxide pathways, their underlying mechanisms differ substantially.

Limitations

This study has several limitations. First, all experiments were conducted under in vitro conditions, which may not fully replicate the complexity of in vivo physiology. Second, while HEK-293 cells are widely used for renal toxicity studies, their embryonic origin and immortalized nature may not accurately reflect the metabolic responses of mature kidney tissue or other organs. As noted by Achilli et al. (33), this cell line offers practical advantages but has translational limitations. Future studies should include additional cell types and in vivo models to better characterize the systemic metabolic effects of Propofol and Thiopental and to explore potential protective strategies.

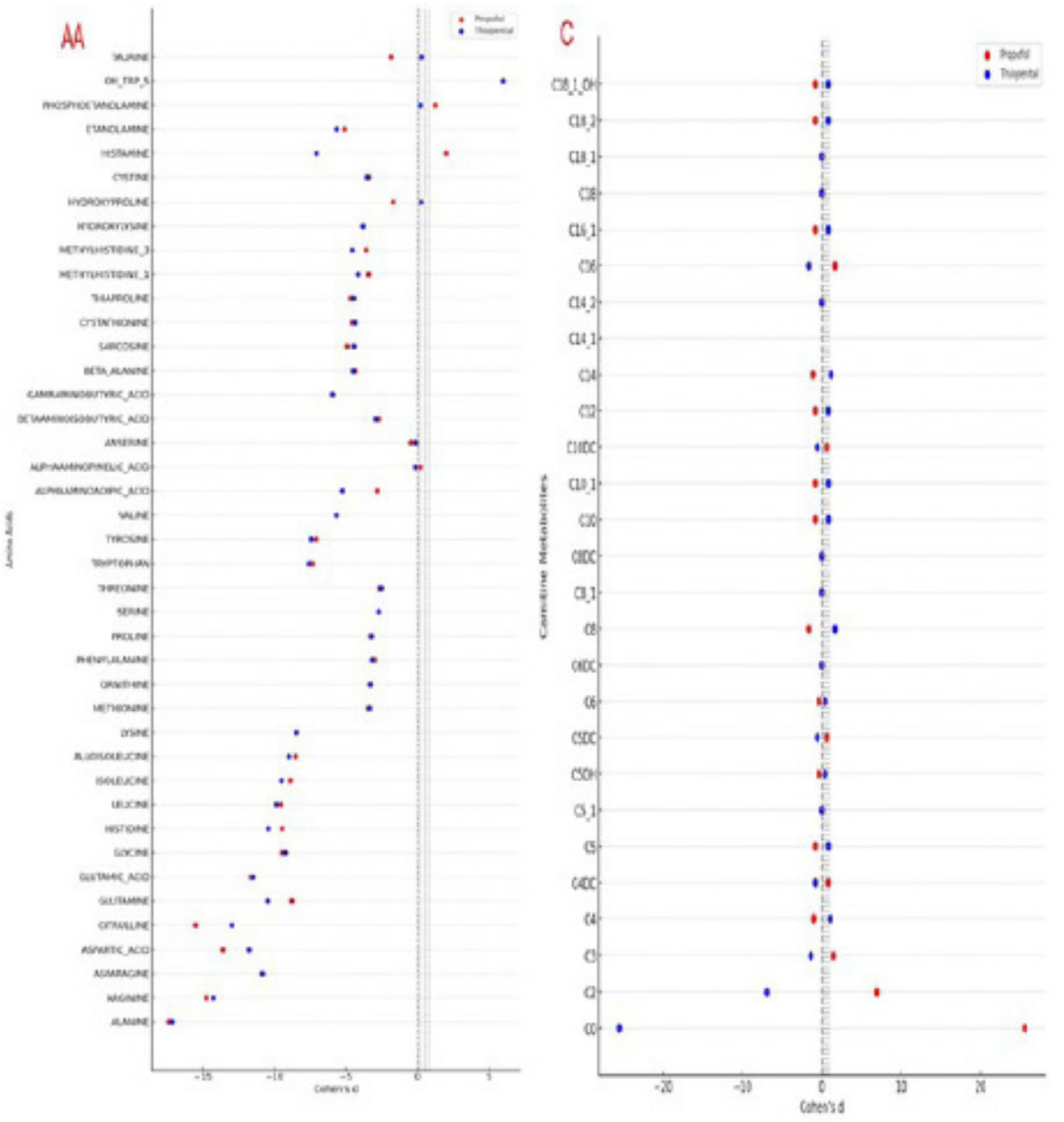

Figure 1. Effect size (Cohen's d) analysis of amino acid and carnitine profile alterations in HEK-293 cells after propofol and thiopental exposure
This dual forest plot displays the effect sizes (Cohen's d) for alterations in intracellular amino acid (AA, left) and carnitine (C, right) levels in HEK-293 cells after exposure to 200 µg doses of Propofol and Thiopental. Red circles represent Propofol and blue circles represent Thiopental. Vertical reference lines denote conventional thresholds for small (d=0.2), medium (d=0.5), and large (d=0.8) effect sizes. Negative values indicate metabolite depletion relative to the control group.

Conclusion

In summary, our findings demonstrate that high-dose exposure to propofol and thiopental induces significant alterations in amino acid and carnitine metabolism in HEK-293 cells, reflecting potential mitochondrial dysfunction and cellular toxicity. Thiopental exhibited a greater cytotoxic potential compared to propofol; however, propofol exerted broader and more profound metabolic disturbances. Specifically, propofol triggered marked depletion of energy-relevant amino acids and severe disruption of carnitine-mediated fatty acid transport, which are consistent with its known ability to elevate ROS levels and compromise mitochondrial respiration. These agent-specific biochemical signatures underscore the critical importance of metabolic profiling in evaluating anesthetic-induced toxicity. The present results may inform the development of targeted strategies to mitigate metabolic side effects, optimize the safety profiles of intravenous anesthetic agents, and guide clinical decision-

making in scenarios involving prolonged or high-dose anesthetic administration.

Ethical Approval: *This study did not require ethical approval as it was conducted entirely on commercially available human cell lines and did not involve human participants or animal subjects.*

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