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Data Article

Dataset on transcriptomic profiling of parenteral nutrition-induced hepatotoxicity in a human liver *in vitro* model

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ABSTRACT

The provided dataset describes the transcriptomic profile of human liver spheroid co-cultures consisting of a human hepatoma cell line (C3A/HepG2 cells) and an immortalized activated human hepatic stellate cell line (LX-2 cells) upon exposure to total parenteral nutrition. High-throughput RNA sequencing was performed using DNBSEQ™ sequencing technology. Following the quality check and filtering of raw sequence reads, the clean reads were aligned to the reference human genome and used to determine differential gene expression. Raw and processed data are deposited in the Gene Expression Omnibus with accession number GSE264357. These data could serve further mechanistic studies on parenteral nutrition-induced liver injury and support translational research on intestinal failure-associated liver disease occurring in individuals receiving total parenteral nutrition.

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Specifications Table

Subject	Toxicology, Hepatology, Bioinformatics
Specific subject area	Food and nutrition-induced liver injury
Type of data	Raw data in the format fq.gz Processed data (table with FPKM values) in the format xls
Data collection	C3A cells, a clonal derivative of the human hepatoma HepG2 cell line (ATCC, Manassas, VA, USA), and LX-2 cells, an immortalized activated human hepatic stellate cell line (Sigma-Aldrich, St. Louis, MO, USA) were cultured according to the manufacturer's instructions and used to generate human liver spheroid co-cultures. After 3 days of culture the spheroid co-cultures were formed and used for exposure to total parenteral nutrition (TPN) mixture (SmofKabiven®, Fresenius Kabi, Kriens, Switzerland) provided by the Department of Clinical Nutrition of the Universitair Ziekenhuis Brussel (Brussels, Belgium), and containing amino acid solution with electrolytes, glucose, and lipid emulsion, trace elements concentrate Addaven® (Fresenius Kabi), and vitamins preparations Vitalipid® Novum Adult and Soluvit® Novum (Fresenius Kabi). Specifically, human liver spheroid co-cultures were exposed to 1 % TPN for 24 h and 144 h, with renewal of cell culture medium every 2–3 days. Untreated human liver spheroids served as control. Total RNA was extracted from approximately 65 human liver spheroid co-cultures per treatment condition and respective controls using the RNeasy Mini kit (Qiagen). 3 independent experiments (exposure and RNA extraction) were performed throughout the study (n=3). Quantification and integrity of RNA samples were measured using the 4150 TapeStation (Agilent Technologies, Santa Clara, CA, USA. Library preparation and RNA sequencing were performed by BGI Genomics (Hong Kong, China), using DNBSEQ™ sequencing technology (DNBSEQ-G400).
Data source location	Department of Pharmaceutical and Pharmacological Sciences, Vrije Universiteit Brussel, Brussels, Belgium.
Data accessibility	Repository name: Gene Expression Omnibus (GEO) from The National Center for Biotechnology Information (NCBI) Data identification number: GSE264357 Direct URL to data: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE264357
Related research article	M. Mihajlovic, S. De Boever, A. Tabernilla, E. Callewaert, J. Sanz-Serrano, A. Verhoeven, A. Maerten, Z. Rosseel, E. De Waele, M. Vinken, Investigation of parenteral nutrition-induced hepatotoxicity using human liver spheroid co-cultures, Archives of Toxicology. DOI: 10.1007/s00204-024-03773-8 10.1007/s00204-024-03773-8

1. Value of The Data

- This data presents a transcriptomic signature of liver cells exposed to total parenteral nutrition, using human-relevant *in vitro* tools in an ethically sound approach.
- The data can assist further translational research in the field of intestinal failure-associated liver disease, especially the research focused on studying underlying molecular mechanisms of disease pathogenesis and progression.
- This data can support the assessment of the robustness of adverse outcome pathways on liver injury (i.e. steatosis) induced by food and nutrients.

2. Background

Parenteral nutrition (PN), a mixture of lipid emulsions, carbohydrates, amino acids, vitamins, minerals, electrolytes, and trace elements, is a life-saving therapeutic tool for various malnourishment-associated conditions and diseases [1]. When intravenously administered PN is the only source of energy, it is defined as total parenteral nutrition (TPN). Even though TPN has

numerous benefits, it can be accountable for various severe adverse effects. One such issue is intestinal failure-associated liver disease, characterized by liver injury which is usually manifested as steatosis, cholestasis, or a combination of both conditions [2]. Although several mechanisms have been proposed, the etiology of hepatic dysfunction associated with TPN administration is still unclear. The use of a human-centered *in vitro* system (liver spheroids co-cultures consisting of hepatocytes and hepatic stellate cells) in combination with high-throughput RNA sequencing and generation of the transcriptomic profile of exposure to TPN can allow for in-depth investigation of TPN-associated liver injury at the mechanistic level. In addition to the primary research article studying the hepatotoxic potential of TPN [3], the hereby presented data can serve to support and complement further mechanistic studies on intestinal failure-associated liver disease pathogenesis and progression.

3. Data Description

The present paper describes the transcriptomic profile dataset of the effect of TPN on human liver spheroids co-cultures over time, deposited in the GEO-NCBI repository (GEO Series accession number GSE264357). During 3 independent experiments, the spheroid co-cultures were exposed to 1 % TPN for either 24 h or 144 h, in order to assess the hepatotoxic potential of TPN [4,5]. The concentration of 1 % TPN was selected based on the cell viability results, as shown in the related research article [3]. The control condition consisted of liver spheroid co-cultures incubated with the cell culture medium solely for 24 h or 144 h. After the incubation of 24 and 144 h, liver spheroid co-cultures exposed to 1 % TPN and respective controls were sampled and RNA was isolated. High-throughput RNA sequencing was performed using DNBSEQ^{TM} sequencing technology, generating thus the raw data containing the sequencing reads and quality scores (information on datasets is indicated in Table 1).

Table 1
Specification of data type, biological conditions and respective dataset names as deposited in the GEO-NCBI repository.

Type of data	Condition	Dataset name
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 24 h, replicate 1	C3A-LX2 spheroids, untreated, 24 h, rep1
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 24 h, replicate 2	C3A-LX2 spheroids, untreated, 24 h, rep2
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 24 h, replicate 3	C3A-LX2 spheroids, untreated, 24 h, rep3
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 24 h, replicate 1	C3A-LX2 spheroids, TPN1 %, 24 h, rep1
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 24 h, replicate 2	C3A-LX2 spheroids, TPN1 %, 24 h, rep2
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 24 h, replicate 3	C3A-LX2 spheroids, TPN1 %, 24 h, rep3
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 144 h, replicate 1	C3A-LX2 spheroids, untreated, 144 h, rep1
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 144 h, replicate 2	C3A-LX2 spheroids, untreated, 144 h, rep2
Raw RNA-seq data	Human liver spheroids co-cultures, untreated 144 h, replicate 3	C3A-LX2 spheroids, untreated, 144 h, rep3
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 144 h, replicate 1	C3A-LX2 spheroids, TPN1 %, 144 h, rep1
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 144 h, replicate 2	C3A-LX2 spheroids, TPN1 %, 144 h, rep2
Raw RNA-seq data	Human liver spheroids co-cultures, exposed to 1 % TPN for 144 h, replicate 3	C3A-LX2 spheroids, TPN1 %, 144 h, rep3
Processed RNA-seq data (FPKM)	All treatment and control conditions as indicated for raw data	GSE264357_core_table_genes_FPKM

Table 2
Statistics of raw sequencing data (reads with unknown base N content, adapter reads, low-quality reads, and clean reads).

Sample	N (%)	Adapter reads (%)	Low-quality reads (%)	Clean reads (%)
Ctrl_144h_n1	0.88	2.62	0.07	96.43
Ctrl_144h_n2	0.90	4.74	0.06	94.31
Ctrl_144h_n3	0.89	3.87	0.06	95.17
Ctrl_24h_n1	0.89	2.70	0.07	96.34
Ctrl_24h_n2	0.86	3.69	0.07	95.38
Ctrl_24h_n3	0.89	2.27	0.07	96.77
TPN_144h_n1	0.87	3.39	0.07	95.68
TPN_144h_n2	0.89	3.33	0.07	95.71
TPN_144h_n3	0.83	3.17	0.07	95.93
TPN_24h_n1	0.85	6.07	0.07	93.01
TPN_24h_n2	0.92	3.14	0.07	95.87
TPN_24h_n3	0.83	4.22	0.07	94.89

N –reads with an unknown base N content; Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

Table 3
Quality statistics of filtered reads.

Sample	Total raw reads (Million)	Total clean reads (Million)	Clean reads Q20 (%)	Clean reads Q30 (%)
Ctrl_144h_n1	47.19	45.5	97.52	92.05
Ctrl_144h_n2	47.19	44.5	97.78	92.92
Ctrl_144h_n3	47.19	44.91	97.73	92.77
Ctrl_24h_n1	47.19	45.46	97.55	92.19
Ctrl_24h_n2	47.19	45.01	97.53	92.12
Ctrl_24h_n3	45.44	43.97	97.53	92.17
TPN_144h_n1	47.19	45.15	97.59	92.3
TPN_144h_n2	47.19	45.16	97.65	92.55
TPN_144h_n3	47.19	45.26	97.57	92.23
TPN_24h_n1	47.19	43.89	97.7	92.71
TPN_24h_n2	47.19	45.23	97.49	92.03
TPN_24h_n3	47.19	44.77	97.72	92.72

Q20 – a quality score of 20 which represents an error rate of 1 in 100 bp and base call accuracy of 99 %; Q30 – a quality score of 30 which represents an error rate of 1 in 1000 bp and base call accuracy of 99.9 %; Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

The statistics of raw data (Table 2) showed that the number of reads with an unknown base N content was less than 1 %, the number of reads containing adapter sequences was around or less than 5 %, the number of low-quality reads was less than 0.1 %, while the clean reads accounted for more than 93 % of total reads.

Quality statistics of filtered reads (Table 3) showed that the total number of clean reads obtained after filtering was on average 44.9 million and that the percentage of quality control Q30 bases was higher than 92 % in all samples, indicating that the quality of sequencing data was adequate for subsequent analysis.

The alignment of clean reads to the reference genome and transcriptome (Table 4) indicated that the rates of uniquely mapping reads were above 90 % and 77 %, respectively; in comparison, the total mapping was above 97 % for alignment to the reference genome and above 82 % for alignment to the transcriptome. Mapping to the reference genome revealed that the insert length was 250–350 bp.

Moreover, Fig. 1 shows each sample's coverage of transcripts by reads, suggesting that nearly 50 % of transcripts exhibit coverage higher than 80 %.

Table 4
Clean reads alignment to the reference genome and reference genes (transcriptome).

Sample	Alignment to reference genome		Alignment to transcriptome	
	Total mapping (%)	Uniquely mapping (%)	Total mapping (%)	Uniquely mapping (%)
Ctrl_144h_n1	98.71	91.16	85.60	81.22
Ctrl_144h_n2	98.25	90.71	83.46	79.06
Ctrl_144h_n3	98.41	90.76	84.59	80.12
Ctrl_24h_n1	98.65	91.20	84.01	79.70
Ctrl_24h_n2	98.42	90.93	83.45	79.12
Ctrl_24h_n3	98.83	91.40	84.93	80.63
TPN_144h_n1	98.48	91.06	82.64	78.14
TPN_144h_n2	98.46	91.08	82.95	78.41
TPN_144h_n3	98.43	90.93	83.12	78.50
TPN_24h_n1	97.89	90.40	82.07	77.63
TPN_24h_n2	98.43	90.97	83.03	78.58
TPN_24h_n3	98.32	90.84	82.85	78.46

Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

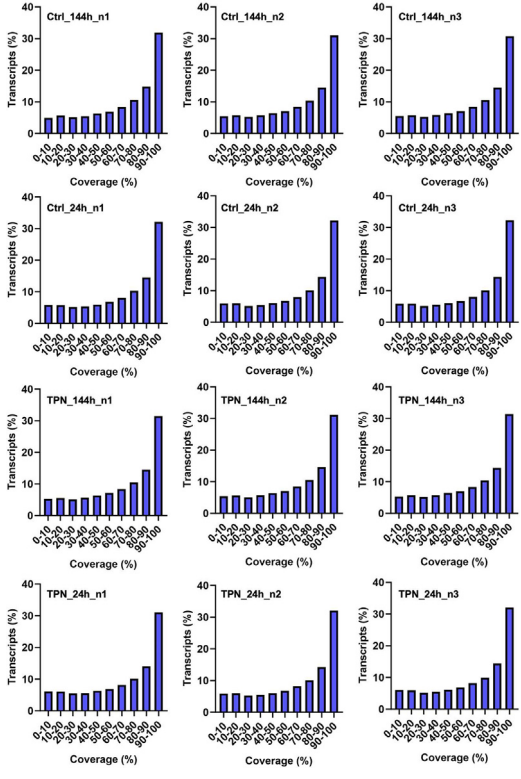

Fig. 1. Percentage of coverage of the transcripts of each sample. The X-axis shows the coverage of transcripts by reads, and the Y-axis represents the proportion of transcripts. Sample identification: Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

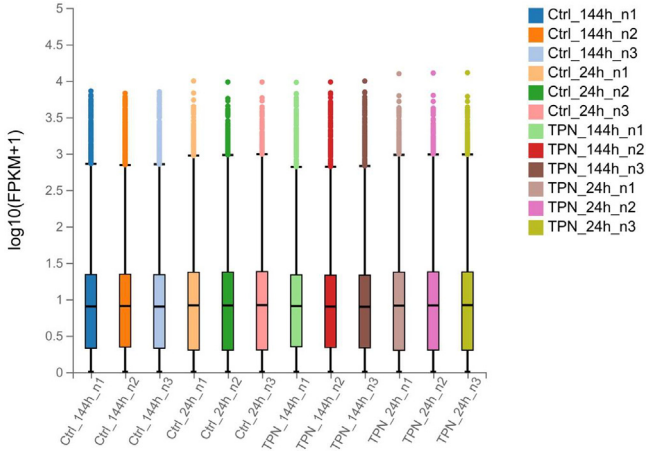

Fig. 2. Gene expression distribution boxplot of each sample based on FPKM values. The X-axis shows the sample name, and the Y-axis displays log10 (FPKM+1). The boxplot for each region has 5 statistics (maximum, upper quartile, median, lower quartile, and minimum). Sample identification: Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

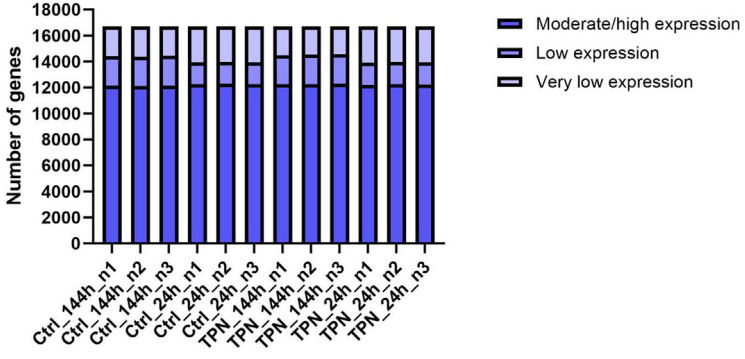

Fig. 3. Classification of the total number of genes of each sample by expression levels based on normalized read counts. The X-axis indicates the sample name, and the Y-axis shows the number of genes. Different colors indicate various expression levels: moderate/high, low, or very low. Sample identification: Ctrl_144h_n1_n2_n3 - Replicates of untreated controls at 144 h; Ctrl_24h_n1_n2_n3 - Replicates of untreated controls at 24 h; TPN_144h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 144 h; TPN_24h_n1_n2_n3 - Replicates of cells exposed to total parenteral nutrition 1 % for 24 h.

In addition to raw RNA-seq data, processed data containing normalized counts of sequencing reads (*i.e.* fragments per kilobase of transcript per million mapped reads - FPKM) is also deposited in the GEO-NCBI repository and provided in the form of a matrix table (dataset information is indicated in Table 1). Boxplots showing the distribution of gene expression levels (FPKM values) and dispersion degree in each sample are shown in Fig. 2.

In the present RNA-seq study, a total of 16.705 genes were detected. Fig. 3 depicts the total number of genes, classified according to the expression levels based on normalized read counts.

4. Experimental Design, Materials and Methods

C3A cells, a clonal derivative of the human hepatoma HepG2 cell line (CRL-10741, ATCC, Manassas, VA, USA), and LX-2 cells, an immortalized activated human hepatic stellate cell line (SCC064, Sigma-Aldrich) were cultured and maintained in Minimum Essential Medium (MEM) (Gibco, Waltham, MA, USA) supplemented with 1 mM sodium pyruvate, 1 % non-essential amino acids (Gibco), 10 % fetal bovine serum (Gibco), 100 U/ml penicillin, and 100 µg/ml streptomycin at 37 °C, at 5 % (v/v) CO_2. Human liver spheroid co-cultures were generated by seeding 500 C3A cells/well and 50 LX-2 cells/well in a total volume of 100 µL in ultra-low attachment (ULA) plates and incubating for 72 h under gentle shaking. After the spheroid co-cultures were successfully generated, the exposure to 1 % TPN was initiated, for either 24 h or 144 h, with regular medium renewal (every 2–3 days of culture). TPN used for cell exposure was derived from a sterile 3-chamber bag of SmofKabiven® parenteral nutrition, containing amino acid solution with electrolytes, glucose, lipid emulsion, trace elements concentrate Addaven®, and vitamins preparations Vitalipid® Novum Adult and Soluvit® Novum (all by Fresenius Kabi, Kriens, Switzerland, and provided by the Department of Clinical Nutrition of the Universitair Ziekenhuis Brussel, Brussels, Belgium). The TPN mixture was prepared according to the manufacturer's instructions. MEM cell culture medium, used to prepare 1 % TPN, was also used for untreated controls at 24 h and 144 hs. Following incubation times of 24 h and 144 h, approximately 65 human liver spheroid co-cultures per condition were pooled, gently disaggregated into single-cell suspension using TrypLE™ Express (Gibco, Waltham, MA, USA) and by pipetting up and down, washed with phosphate-buffered saline (PBS), and centrifuged at 1500 rpm for 5 minutes at 4 °C to obtain a single-cell pellet. Afterward, cell pellets were used to extract total RNA employing the RNeasy Mini kit (Qiagen) and by following the manufacturer's instructions. Subsequent steps leading to and including RNA sequencing were performed by BGI Genomics (Hong Kong, China). Firstly, the RNA samples were quantified and checked for quality using the 4150 TapeStation (Agilent Technologies, Santa Clara, CA, USA); all RNA samples were of high quality and therefore eligible for sequencing (RNA Integrity Number [RIN] for all samples was comprised between 9.7 and 10). After mRNA enrichment, using oligo(dT) magnetic beads, mRNA was fragmented and first-strand cDNA was generated, followed by a second-strand cDNA synthesis using dUTP. The synthesized cDNA was subjected to end-repair, 3' adenylation, and adapter ligation, which was followed by uracil-DNA-glycosylase-mediated degradation of the dUTP-marked strand and PCR amplification of the remaining strand. The double-stranded PCR products were heat-denaturated and circularized through splint oligo ligation, generating the single-strand circle DNA which was formatted as the final library. Following library preparation, validation, and amplification with Phi29 DNA polymerase to create DNA nanoballs, sequencing was performed using the DNBSEQ-G400 platform, generating around 22.5 million (paired-end 150 bp) reads per sample. Sequencing reads were filtered to remove low-quality, adaptor-polluted and unknown base reads using SOAPnuke software (v1.5.2; BGI Genomics) [6]. After filtering, the clean reads were mapped to the reference human genome assembly GRCh38.p13 (version GCF_000001405.39_GRCh38.p13) using HISAT2 (v2.0.4) [7]. Clean reads were mapped to reference transcripts using Bowtie2 software (v2.2.5), and the gene expression level of each sample was calculated using RSEM (v1.2.8) [8,9]. Differential gene expression (DEG) analysis was performed using the DESeq2 analysis method, with normalized gene expression levels represented as FPKM [10,11]. Only transcripts with a log2-transformed gene expression fold change $\log_2|FC| > 0.58$ and adjusted p-value (Q value) < 0.05 were deemed significantly differentially expressed.

Limitations

None.

Ethics Statement

The authors declare that they have read and followed the ethical requirements for publication in *Data in Brief* and confirm that the current work does not involve human subjects, animal experiments, or any data collected from social media platforms.

Data Availability

Investigation of parenteral nutrition-induced hepatotoxicity using human liver spheroid co-cultures (Original data) (Gene Expression Omnibus (GEO)).

CRediT Author Statement

Milos Mihajlovic: Conceptualization, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization; **Mathieu Vinken:** Conceptualization, Writing – original draft, Writing – review & editing, Funding acquisition, Supervision.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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