The impact of cell culture and copper dose on copper trafficking genes in bovine liver.

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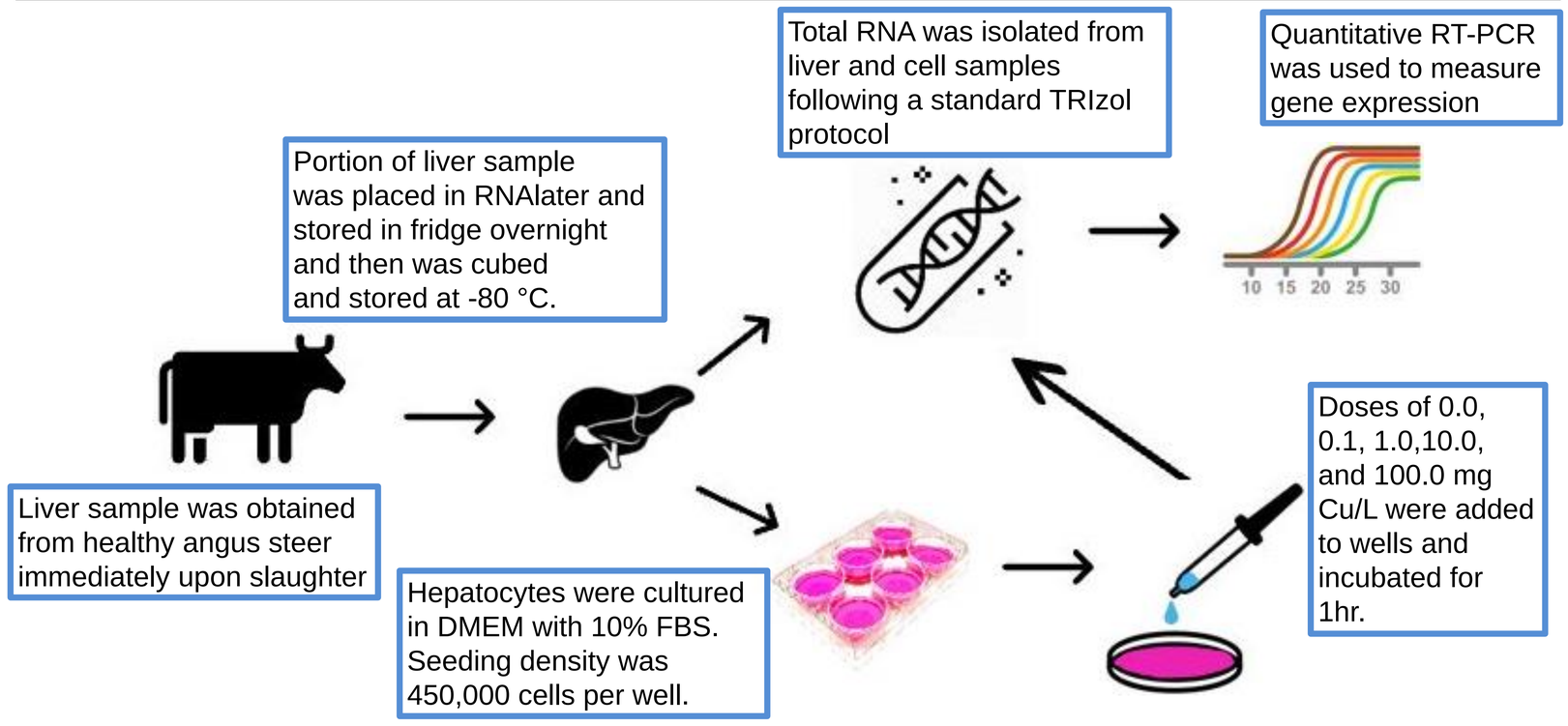
ABSTRACT

The objective of the current experiment was to investigate the influence of Cu dose on the relative abundance of Cu trafficking genes in cultured bovine hepatocytes. A liver sample was obtained immediately post-mortem from one healthy Angus steer. Hepatocytes were isolated, counted, and seeded at equal density into 15 separate wells, and incubated for 1 hour in culture media containing: 0.0, 0.10, 1.0, 10.0, or 100 mg Cu/L (3 replicates per Cu dose). Following incubation, cells were collected, and total RNA was isolated. Quantitative RT-PCR was used to determine the abundance of transcripts for proteins involved in Cu homeostasis. The identified targets were: ALDH2, APOA1, ATOX1, ATP7A, ATP7B, BHMT, BLVRB, CA2, CCS, COX17, CTR1, ELN, GAPDH, GLUD1, GSS, LOXL1, PDIA3, SOD1, SOD3. β-Actin (ACTB) served as the endogenous control. Significant linear responses existed for ALDH2 ($P < 0.001$), ATOX1 ($P < 0.01$), PDIA3 ($P < 0.05$). As Cu dose increased, the relative abundance of ALDH2 increased, and ATOX1 and PDIA3 decreased. Significant quadratic responses existed for ATP7B ($P < 0.001$), COX17 ($P < 0.05$), and SOD1 ($P < 0.05$). The relative abundance of COX17 was lesser at 0.1 and 1.0 mg Cu/L when compared to 0.0, 10, and 100 mg Cu/L. Transcript abundance for ATP7B and SOD1 was lower at 0, 1, and 100 mg Cu/L when compared to 0.1 and 10 mg Cu/L. These data indicate that certain transcripts are differentially expressed in cultured bovine hepatocytes in response to increasing Cu dose.

MATERIALS AND METHODS



RESULTS AND DISCUSSION

Figure 1: Log transformed relative abundance of cell cultured bovine hepatocytes compared to whole liver

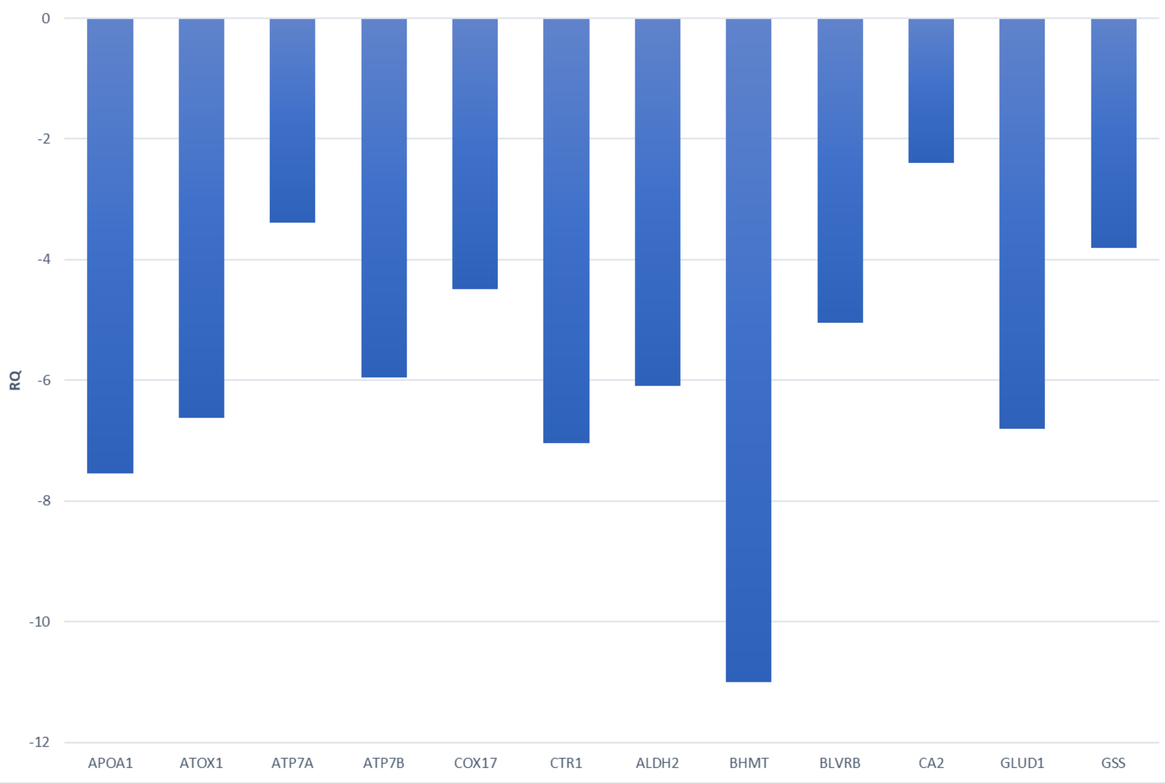


Figure 2: Influence of copper dose on log transformed relative abundance of copper homeostatic genes in cultured bovine hepatocytes

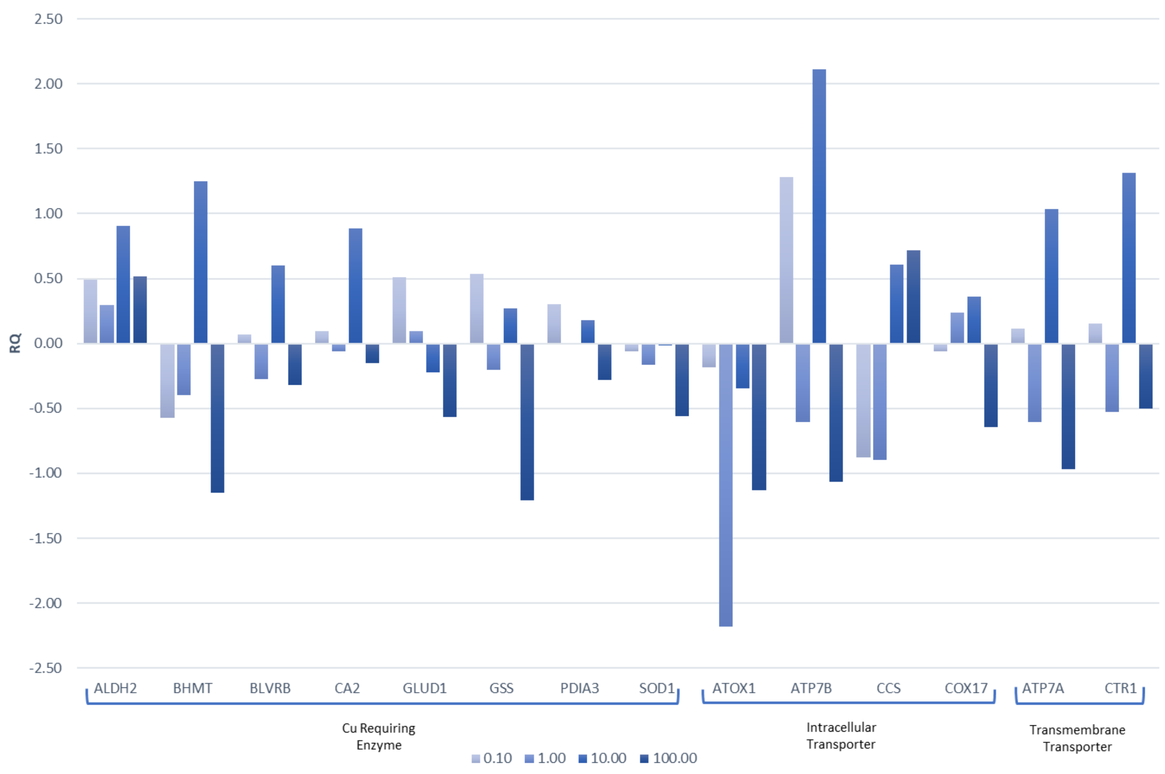


Table 1. The effects of copper dose on the change (Δ) in threshold (C_T) of copper homeostatic genes in cultured bovine hepatocytes.

Item	Treatment (ppm Cu)					SEM	P<	Linear P<	Quadratic P<	Cubic P<
	0	0.1	1.0	10.0	100.0					
ALDH2	4.06	3.57	3.76	3.15	3.54	0.107	0.002	0.002	0.04	0.37
ATOX1	6.36	6.54	6.58	6.72	7.46	0.196	0.05	0.006	0.14	0.28
ATP7A	7.93	7.82	8.54	6.90	8.90	0.174	0.001	0.10	0.02	0.001
ATP7B	9.46	8.20	10.06	7.34	10.50	0.224	0.001	0.13	0.001	0.005
BHMT	10.12	10.69	10.51	8.87	11.26	0.291	0.002	0.62	0.08	0.001
BLVRB	3.85	3.78	4.13	3.25	4.18	0.107	0.001	0.74	0.09	0.003
CA2	8.64	8.55	8.71	7.76	8.79	0.107	0.001	0.18	0.02	0.001
CCS	10.87	11.94	11.91	10.45	10.30	0.293	0.03	0.04	0.02	0.05
COX17	5.55	5.78	5.48	5.36	6.36	0.117	0.002	0.02	0.005	0.002
CTR1	7.79	7.66	8.31	6.49	8.27	0.161	0.001	0.69	0.06	0.001
GLUD1	6.22	5.71	6.12	6.44	6.78	0.119	0.001	0.001	0.005	0.04
GSS	9.69	9.15	9.89	9.41	10.89	0.292	0.020	0.02	0.03	0.48
PDIA3	3.44	3.14	3.45	3.26	3.66	0.080	0.020	0.08	0.02	0.91
SOD1	4.90	4.96	5.07	4.91	5.46	0.097	0.012	0.006	0.08	0.06

- Figure 1 shows the expression of Cu homeostatic genes *in vitro* relative to their abundance in an *in vivo* liver sample. RNA extracted from cultured hepatocytes is much lower but still detectable after 2hrs in culture.
- Figure 2 shows a Cu dose response across treatments on cell cultured bovine hepatocytes. Fourteen Cu influenced genes were selected for this portion of the experiment and have been grouped by protein function for the corresponding gene. These data suggests that within intracellular transporters, gene expression does not follow a dose dependent response. Within Cu requiring enzymes, all genes were upregulated by at least one Cu dose. Within transmembrane transporters, both genes followed similar trends in upregulation of gene expression at two Cu doses.
- Table 1 shows the effects of Cu dose on the change in the threshold of Cu homeostatic genes in cultured bovine hepatocytes. All fourteen Cu homeostatic genes, ALDH2, ATOX1, ATP7A, ATP7B, BHMT, BLVRB, CA2, CCS, COX17, CTR1, GLUD1, GSS, PDIA3, and SOD1, were impacted ($P < 0.05$) by Cu dose.
- Future research is scheduled to investigate these genes in liver samples from live animals that have been fed different doses of Cu.

CONCLUSION

These data suggest that:

- Cu homeostatic gene expression is altered by the process of culturing hepatocytes.
- Cu homeostatic gene transcript abundances are altered in response to different Cu concentrations in culture.

REFERENCES

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