universität wien

DIPLOMARBEIT / DIPLOMA THESIS

Titel der Diplomarbeit / Title of the Diploma Thesis

„The Effects of L-Carnitine as Nutrigenomic Factor on Gene Expression Under Various Conditions. “

verfasst von / submitted by

Magdalena Steiner

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2019 / Vienna, 2019

Studienkennzahl lt. Studienblatt / degree programme code as it appears on the student record sheet:	A 190 445 456
Studienrichtung lt. Studienblatt / degree programme as it appears on the student record sheet:	Lehramtsstudium UF Biologie und Umweltkunde UF Geographie und Wirtschaftskunde
Betreut von / Supervisor:	Ass.-Prof. Mag. Dr. Reinhold Hofbauer

Declaration

I declare,

- that I have authored this thesis independently,
- that I have not used other than the declared sources and resources
- and that I have explicitly marked all material which has been quoted either literally or by content from the used sources.

Vienna,

18.02.2019 

Erklärung

Hiermit versichere ich,

- dass ich die vorliegende Diplomarbeit selbstständig verfasst, andere als die angegebenen Quellen und Hilfsmittel nicht benutzt und mich auch sonst keiner unerlaubter Hilfe bedient habe,
- dass ich dieses Diplomarbeitsthema bisher weder im In- noch im Ausland in irgendeiner Form als Prüfungsarbeit vorgelegt habe
- und dass diese Arbeit mit der vom Begutachter beurteilten Arbeit vollständig übereinstimmt.

Wien,

18.02.2019 

Acknowledgment

I would like to thank various people for their contribution to this project:

First of all Ass.- Prof. Mag. Dr. Reinhold Hofbauer, for his willingness to guide me the last two years. He introduced me in the department of medical biochemistry and followed the whole project with patience.

I also want to thank the whole research group and my predecessors, for their advice, their groundwork and their enrolment.

Finally and especially I would like to thank my family and friends, for their never ending encouragement throughout the process, their motivating advice and their effort to make me keep on going.

Abstract (English)

BACKGROUND: L-carnitine as important nutrigenomic metabolite that has an enormous impact on gene expression.

OBJECTIVE: The objective of this research was to compare the gene expression of the genes BCOX1, TBDN100, STK4 and LMNB1 in two different cell lines, originating from muscle and liver cells. These cell lines were cultivated under different L-carnitine supplementation levels. On one hand we have chosen the liver cell line WRL 68, which represents the metabolic function of the liver. On the other hand we analysed the SKMC cell line. It represents an established skeletal muscle cell line with a storage function for L-carnitine.

MATERIALS/METHODS: All cells intended for L-carnitine supplementation were precultivated in the laboratory for three days in a carnitine deficient cell culture medium containing 10% dialysed FCS, and then induced by addition of the desired L-carnitine amount for four hours. The mRNA was extracted and transcribed into cDNA. By conducting the experiments, qPCR was the method of choice to detect the steady state gene expression levels.

RESULTS: All examined genes were significantly influenced by L-carnitine regarding their amplification rate. There was a significant difference between the gene expression of TBDN100 in the WRL68 liver cells and the SKMC cells. It is notable, that the gene expression of TBDN100 showed downregulation correlated to the amount of L-carnitine induction in the SKMC. Another important aspect was the significant positive correlation between BCOX1 and the 40µM L-carnitine supplementation level during cultivation in cell culture.

CONCLUSION: Different concentration levels of L-carnitine in liver and muscle cells influence the metabolism in humans in general as well as the gene expression of the four chosen genes in particular. TBDN100 became apparent as the most divergent gene in the comparison of liver and skeletal muscle cells. The expression of this gene is important for the process of protein modification after translation and the acetylation of histones.

Abstract (German)

HINTERGRUND: L-Carnitin hat als wichtiger nutrigenomischer Metabolit, einen enormen Einfluss auf die Genexpression.

ZIELE: Das Ziel dieser Arbeit ist die Herstellung eines Vergleiches zwischen Genexpressionen der Gene BCOX1, TBDN100, STK4 und LMNB1 in zwei verschiedenen Zelllinien. Diese Zelllinien stammen in unserem Fall aus dem Muskel und aus der Leber und wurden mit diversen L-Carnitin-Zusätzen kultiviert. Zum einen haben wir dafür die humane Leberzelllinie WRL68 ausgewählt, die morphologisch Hepatozyten sowie primären Leberzellen entspricht und somit die metabolischen Funktionen der Leber repräsentiert. Zum anderen wurde dazu vergleichend die SKMC Zelllinie analysiert, die ursprünglich von primaren humanen Skelettmuskelzellen isoliert wurde (e.g. Musculus pectoralis major) und somit optimal die Speicherfunktion der Skelettmuskelzellen darzustellen vermag.

MATERIAL/METHODEN: Alle Zellen, welche für den Zusatz von L-Carnitin vorgesehen waren, wurden vorher für drei Tage in einem L-Carnitin-freien Medium, mit 10%-igem dialysierten FCS kultiviert. Anschließend wurde die gewünschte Menge L-Carnitin beigefügt und die Zellen für weitere vier Stunden kultiviert. Die mRNA wurde extrahiert und in cDNA transkribiert. Als Methode der Wahl, zur Feststellung der Mengen an exprimierter Gene, diente die qPCR.

ERGEBNISSE: Alle untersuchten Gene zeigten einen signifikanten Einfluss von L-Carnitin bezüglich ihrer Amplifikationsrate. Es gab einen signifikanten Unterschied zwischen der Genexpression von TBDN100 in den WRL68 Leberzellen und den SKMC Zellen. Hervorzuheben ist, dass die ansteigenden Mengen der L-Carnitin-Zugabe in den SKMC die Genexpression von TBDN100 entsprechened herunter regulieren konnte. Ein weiterer wichtiger Aspekt ist die signifikant positive Korrelation zwischen BCOX1 und der 40µM L-Carnitinsupplementation als Kultivationsbedingung in der Zellkultur.

SCHLUSSFOLGERUNG: Unterschiedliche Konzentrationsstufen von L-Carnitin in den Leber- und Muskelzellen beeinflussen den Metabolismus des Menschen generell

und die Genexpression der vier ausgewählten Gene im Speziellen. TBDN100 hat sich als das variabelste Gen im Vergleich zwischen Leber- und Skelettmuskelzellen herausgestellt. Die Expression dieses Gens spielt eine wichtige Rolle bei der Modifikation der Proteine nach der Translation und der Azetylierung von Histonen.

Index

List of Figures

Table Index

1. Introduction

1.1. Aims of Thesis

This thesis is mainly investigating the effects of L-carnitine, as nutrigenomic factor, on gene expression in different cell lines as model systems. On one hand there is the liver cell line WRL68, which represents the metabolic function of the liver. On the other hand we have chosen the skeletal muscle cell line SKMC. It represents the storage function of skeletal muscle cells, with regard to L-carnitine this is a major function of muscle cells. Aim of the project was to compare the gene expression in these two cell lines, influenced by different L-carnitine supplementation levels. Once transcribed to cDNA the steady state mRNA levels of the target genes can be directly analysed by qPCR. The question, whether and which amount L-carnitine regulates the steady state mRNA levels of the target genes in the cell lines differently or not, is the key issue. A microarray analysis has revealed that L-carnitine is a regulating metabolite of gene expression of hundreds of genes.(1,2) Four genes of this microarray analysis have been selected for the investigation based on their presumed pivotal impact on the genomics of important cell and tissue specific body functions. Those genes are BCOX1, TBDN100, STK4 and LMNB1. The cell lines mentioned above were cultivated in our laboratory. All cells intended for L-carnitine supplementation were pre-cultivated for three days in a carnitine deficient cell culture medium containing 10% dialysed FCS, and then induced by addition of the desired L-carnitine amount (0µM, 40µM, 80µM) for four hours.(3,4) The experimental process was performed with Real Time PCR and the use of SYBR Green.

1.2. Biosynthesis

L-carnitine (3-hydroxy-4-trimethylaminobutyrate) is an important metabolite synthesised from the carbon chain and nitrogen atom of lysine and the methyl groups of methionine. Lysine and methionine are essential amino acids, which are mainly taken up by nutrition and accumulate in muscles. According to studies with rats, there were no L-carnitine deficiency symptoms by reduced intake of lysine. It is assumed that less than 1% lysine is needed for synthesis of L-carnitine. The compound is mainly synthesised in the liver. Vitamin C as well as ferrous ions and niacin have an impact on the synthesis process.(5-7)

The highest rates of carnitine concentrations are located in heart and skeletal muscle cells. The intermediate 6N-trimethyl-L-lysine is a product of protein turnover and is contained in histones, actin, myosin and calmodulin.(5)

Skeletal muscles are presumed as place of synthesis of 6N-trimethyl-L-lysine.The synthesis of 6N-trimethyl-L-lysine for creation of L-carnitine starts with a hydroxylation catalysed by 6N-trimethyl-L-lysine dioxygenase. This dioxygenase is found in mitochondria of tissues like liver, kidney, heart, skeletal muscle, and brain. The resulting product of the step before is 3hydroxy-6N-trimethyl-L-lysine, which splits up in glycine and 4-trimethylaminobutyraldehyde, catalysed by 3hydroxy-6N-trimethyl-L-lysine aldolase. After this step it is oxidised to 4-N-trimethylaminobutyrat by 4-trimethylaminobutyraldehyde dehydroxygenase. The last step is a hydroxylation performed by gamma-butyrobetaine dioxygenase and yields to L-carnitine.(5,8)

1.3. Carnitine Shuttle Mechanism

The main function in the human metabolism is the transfer of long chain fatty acids into the mitochondria. There the ß-oxidation takes place.(7,9) A very important further function of L-carnitine is to balance the ratio between acetyl CoA and CoA and the local storage for acetylCoA by providing acetyl-Lcarnitine. (8,10-12) Short and medium chain fatty acids pass the mitochondrial membrane immediately, while long chain fatty acids need to be transferred to get through the membrane as carnitine-esters. Acyl-CoA can't pass the inner mitochondrial membrane.(6) For this purpose there are several enzymes which catalyse this transfer and this process is called carnitine shuttle mechanism. Acyl-CoA-synthetase activates the long chain fatty acids. A compound is built of coenzymA and the fatty acid, called acyl CoA.(5,9) Then the lipid metabolite is transesterified in fatty acyl carnitine by L-carnitine palmitoyltransferase I. This step enables to pass the carnitine-acylcarnitine translocase at the inner membrane of the mitochondria.(5,13) This enzymatic reaction turned out to be the rate-limiting step of the transport of long chain fatty acids into mitochondrial matrix.(10,14,15)

In the mitochondria finally, the acyl group is transferred back to the coenzymA and can be used for ß-oxidation. This step is catalysed by the enzyme L-carnitine palmitoyltransferase 2.(5) At the same time L-carnitine serves here as a back-up system for the energy rich acetylCoA.(6) The rate of long-chain acylcarnitines has pivotal effects on the mitochondrial lactate and pyruvate metabolism.(15,16)

L-carnitine returns back to the cytosol outside of the mitochondria and receives some new long chain fatty acids and the cycle starts over again. This mechanism emphasises the importance of L-carnitine for the human body and it's energy supply by ß-oxidation.(7) There are many possibilities this metabolic pathway can be affected.

1.4. L-carnitine and Metabolism

It has to be noted that L-carnitine can be synthesised of the body by itself to some extent, dependent on age and nutritional state, on the other hand it has to be taken up from nutrients, mainly meat.(5,9,17) While a small amount of L-carnitine is synthesised in the liver and the kidney, it's mostly accumulated in skeletal muscles. The major part of the L-carnitine pool of the body is stored in skeletal muscles. In other tissues the uptake goes along with transporters.(5,6,15,18,19) The plasma level of L-carnitine is low, therefore it needs a transporter and carrier to pass the cell membrane. The most significant transporter is the organic cation transporter 2 (OCTN2). (15,18,19) Primary carnitine deficiencies are linked to a mutation in the OCTN2 transporter.(19,20) The OCTN2 has a limited L-carnitine absorption capacity. The gene expression of OCTN2 decreases with progressing age.(21) L-carnitine takes part in the reconstruction of phospholipids of the membrane of erythrocytes and the consumption of ketone bodies.(22)

It is known for its antioxidant, anti-inflammatory, anticytokine, and anti-osteoporotic features.(23) In so far L-carnitine normalises the redox state in brain and increases urea synthesis, which leads to better outcome in acute ammonium intoxication.(5,24) Circulating L-carnitine in the bloodstream leads to urine elimination of it.(15,25)

As mentioned before L-carnitine is an antioxidant, and as such it helps against oxidative stress, which is assumed to be an important factor in aging.(26) Impairment of the carnitine cycle or ß-oxidation can lead to releasement of fats from the adipose tissue during fasting. These fats accumulate in skeletal muscles, liver and heart. The results are steatosis and decreased production of ketones. Ketones provide an alternative energy source next to glucose.(27)

1.4.1. Heart

The concentration level of L-carnitine in cardiac cells is much higher than in plasma.(28) Carnitine accumulates in the cardiac tissue, especially in the region of the left ventricle. While there is a deposition of carnitine in the heart, no synthesis of the compound is located there.(29-31) Cardiac and skeletal muscle cells profit especially from the energy uptake through long chain fatty acids.(15,32) Propionyl-L-carnitine and acetyl-L-carnitine have a positive influence in reducing noxious metabolites from coronary thrombosis or embolism.(33) Protective consequences of the intake of L-carnitine are decreased ventricular dysfunction and decreased arrhythmia. (34) The mutation of an organic cation transporter 2 gene impairs the ß-oxidation in cardiac cells.(35)

1.4.2. Brain and Nerves

A study about peripheral nerve regeneration points to the fact that L-carnitine locally administered or given systematically, enhances the healing process in the nerve fibres.(36) L-carnitines neuroprotective effects can be attributed to its anti-apoptotic features as well as its anti-inflammatory mechanisms. It stabilises mitochondrial membranes and as a result of this it has antiradical and antioxidative effects.(37)

1.4.3. Muscles

Skeletal muscles are the main storage of the body for L-carnitine. (18,19) According to Sakurauchi et. al., muscle cramps can be improved by supplementation of L-carnitine.(38) This should be helpful especially for people with diabetes mellitus or cirrhosis.(32,39) Exercise can lead to local hypoxia due to impairment of the citric cycle. This might lead to inflammation and muscle injury. Reactive oxygen species might be generated. Inflammation influencing factors might cause delayed onset muscle soreness. Hypoxanthine, creatine kinase and methylenedioxyamphetamine are key metabolites in this occurrence.(40,41)

1.4.4. Immune System and Antioxidants

As mentioned before L-carnitine has a role as apoptotic inducer and decreases antiproliferative effects, caused by interleukines.(42) L-carnitine is an antioxidant, and as such it helps against oxidative stress, which is assumed to be an important factor in aging and during exercise recovery.(26,41)

1.4.5. Liver

A small amount of L-carnitine is synthesised in the liver. (15) One consequence of HCV is steatosis. There is a relationship between steatosis and reduced function of ß-oxidation and therefore with L-carnitine as well.(43,44)

1.4.6. Fertility

Another effect of L-carnitine is the improvement in the management of infertility.(42,45,46) The positive antioxidative effects on free radicals improve blastocyst rates. L-carnitine induces apoptosis and initiates a decrease of antiproliferative effects. All these factors affect embryogenesis. For men L-carnitine influences the sperm motility and maturation.(42)

1.4.7. Kidney

The kidney is an important organ, which influences homeostasis by variable tubular reabsorption.(19) Chronic hemodialysis affects this homeostasis and leads to carnitine disordered symptoms.(18)

1.4.8. Bones

A study of ovariectomised rats has shown improvement in osteoporosis issues by L-carnitine supplementation. Estrogen results in a decreased bone resorption by inhibiting osteoclasts and suppressing synthesis of cytokines of osteoblasts.(47-49) This leads to the conclusion that estrogen has an decreasing effect on inflammation.(50-53) Age-induced inflammation processes and oxidative stress impairs energy metabolism in mitochondria and leads to an accumulation of acylCoA in the matrix of the mitochondria. L-carnitine acts as carrier for acylCoA.(54) Further on it provides long-chain fatty acids as an energy ressource for bone cells.(55) All of this points to the fact that L-carnitine has a bone protective effect in postmenopausal women.(23,48)

1.4.9. Homeostasis

There is a homeostasis between acyl-L-carnitine and free L-carnitine. The kidney is an important organ, which influences this homeostasis by variable tubular reabsorption.(19) Further regulating factors of the homeostasis are bioavailability, urinary excretion and the transport.(41) Homeostastis and tissue distribution of L-carnitine alters by advanced age.(41,56)

1.4.10. Endocrinology

The intake of L-carnitine has reducing effects on serum leptin in diabetic patients.(57) Hypothyroidism is an ordinary disease which needs appropriate therapy to avoid side effects. Fatigue is one side effect of hypothyroidism, caused by impairment of the energy metabolism. It has already been shown that L-carnitine enhances fatigue symptoms due to different diseases like cancer, chronic kidney disease or chronic hepatitis.(58-62) All these facts point towards a promising approach of L-carnitine as therapy option.

1.5. Carnitine Deficiency

Carnitine deficiencies caused by genetical mutations, certain diseases or even malnutrition can cause lipid metabolism disorders and cardiovascular diseases.(6,63) Carnitine deficiencies are rather consequences of abnormalities than the cause by itself.(9) It can be linked to atherosclerosis.(18,64) Acetyl-L-carnitine deficiency is correlated to depressive disorders.(65) The main problem of carnitine deficiency is the impaired transport of fatty acids.(66) Primary carnitine deficiencies are linked to a mutation in the OCTN2 transporter.(19,20)

1.6. Carnitine Supplementation

Supplementation of L-carnitine in combination with exercise is a diverse subject. There are several possible factors which have a pivotal impact on studies and trials. Some of those are the fitness level, the supplementation period and the testing itself of the test subject. So there is a difficulty for generalisation. (41,67-69) L-carnitine – supplementation is a common way to compensate deficiencies. It also helps reducing mortality of angina or ventricular arrhythmia in myocardial infarcts as well as progressing type 2 diabetes mellitus.(34,70) Further L-carnitine addition shows reduction of fatigue-symptoms by hypothyroidic patients.(62) The most common intake of metabolites orally for carnitine production are trimethylamine and gamma-butyrobetaine.(9,71) The oral intake of L-carnitine shows low bioavailability.(18) According to Rebouche the activity of gamma-butyrobetaine hydroxylase differs with human's development.(5) Children´s hepatic enzymes have a far more limited activity than ones of adults. This leads to the hypothesis of a reduced function of gamma-butyrobetaine hydroxylase and with it a decreased carnitine function in human infants. Regardless of whether adults or infants got gamma-butyrobetaine supplementation, both showed an increasing carnitine level, which disproves the hypothesis mentioned before.(5) Further, it turned out that the rates of N-trimethyl-L-lysine is linked to the rates of the biosynthesis of carnitine. Studies have shown that supplementation of either N-trimethyl-L-lysine or gamma-butyrobetaine are followed by an increased rate of carnitine.(5) The supplementation of high doses of L-carnitine as therapy option for uremic patients enables a better hemodialysis tolerance and with it the outcome of several heart diseases improves.(72)

L-carnitine supplementation seems to enhance the blood flow by influencing the endothelial function independent of energy production or muscle L-carnitine accretion.(41,73,74) Stress markers increased by exercise can be reduced.(41) Adverse effects of oral L-carnitine supplementation were described, when being converted to trimethylamine (TMA) by intestinal microbiota, which is further metabolised to trimethylamine-N-oxide (TMAO). The latter having been shown to be involved in the promotion of atherosclerosis in animal models. (75) In L-carnitine supplementation studies with human test persons, this metabolite was converted into the atherosclerosis- and thrombosis-promoting metabolite TMAO by gut microbiota. (76) The latter pointing towards a real danger when patients have to receive oral L-carnitine supplementation.

1.7. L-carnitine as Nutrigenomic Factor

L-carnitine not only is key metabolite it also has great influence on metabolic effects in the human body, by directly influencing the genetics. L-carnitine therefore is a nutrigenomic factor. A microarray analysis performed in our laboratory did reveal that L-carnitine is regulating gene expression by increasing as well as decreasing the mRNA levels of hundreds of genes.(2)

1.8. Genes

1.8.1. BCOX1

BCOX1 represents an alternative spliced version of the KIAA0100 gene. It is expressed in ordinary placenta and pancreas tissue.(77,78) Research has shown that BCOX1 is overexpressed in breast and prostate carcinomas, and lymph node metastases.(79,80) Overexpression of BCOX1 in prostate carcinomas could be inhibited by microRNA-195. This was accomplished by binding of miR-195 to the 3'UTR of BCOX1.(79) According to Cui et al. the donwregulation of KIAA0100 inhibited the cell proliferation and had an apoptotic effect in the U937 cells.(81) These properties might lead to enhancement in immunotherapy for acute monocytic leukemia. Besides it was assumed that it is a part of the mitochondrial genome.(81) Another theory argued for BCOX1 to provide the ability to modulate the aggressive behaviour of cancer cells through heat shock proteins and microtubule.(82) BCOX1 was assumed to be located in the cytoplasm.(77,78) Since it was also detected by the previously discussed microarray analysis about L-carnitine regulation, the KIAA0100 gene was chosen for a closer study in this investigation.(2)

1.8.2. TBDN100

The transcriptional coactivator tubedown-100 also known as N-alpha-acetyltransferase 15 (NATH) encodes for a protein which is part of the protein N-acetyltransferase complex.(83) The second part of this complex is the protein hARD1. Both of them are expressed in promyelocytes, glioma and epithelial cells.(84) As a complex, they catalyse the process of protein modification after the translation, by transferring an acetyl group to the amino group of the forming protein.(85) Acetylation of histones plays a key role in transcriptional dysregulation.(86) Downregulation and proliferation are resulting effects and thereby NATH gained more importance in tumor cells.(83) It is overexpressed in papillary thyroid carcinomas.(87) This knowledge was used as a basis for developing tumor therapies.(86) Further on the two compounds of this complex are highly expressed during brain development in places of cell migration and

division, but get downregulated in a postnatal state of neurone differentiation.(88-90) There was a correlation detected between downregulation of NATH and increased age in several tissues, like the nervous system and the heart.(90) The highest rates of NATH-expression were found in a Burkit lymphoma cell line and in testis.(87) Impairment of this important complex might have pivotal effects on a organisms development due its various gene expression.(90) There were various mutations of NATH detected in researches of patients with autosomal dominant mental retardation-50. Haploinsufficiency of this gene turned out to be the most frequent reason for this disorder.(90) In conclusion, NATH became apparent in oncogenesis, apoptosis and cell cycle regulation.(84) In a microarray analysis NATH turned out to be regulated by L-carnitine.(2,91)

1.8.3. LMNB1

This gene encodes for lamin protein B1, that is part of a network of proteins, which is significant for structural and morphological properties of the nucleus and the regulation of chromosome functions.(92-94) Chromatin structures were proven to get changed by it and this was important for spindle assembly.(95,96) In the capacity of these functions, LaminB1 had shown to encourage the preservation of the nuclear envelope in male reproductive cells after meiosis.(97) Antibodies against LMNB1 have been correlated with autoimmune diseases. Duplication of this gene led to increased expression levels of Lamin B1 in lymphoblasts and is associated with autosomal dominant leukodystrophy (ADLD).(98,99) It is a disease of the central nervous system generated by a loss of myelin. Overexpression of this protein in cultured cells showed an altered nuclear morphology (98) LMNB1 serves as biomarker for early stages of a hepatocellular carcinoma and senescence. Its expression levels could be correlated with tumor stages.(100) And finally L-carnitine was documented to influence Lamin B1 expression levels.(2,91)

1.8.4. STK4/MST1

MST1 encodes for serine/threonine kinases which influence cell proliferation, viability, structure and motility.(101) These kinases react to enormous cellular stress and are known as factor in programmed cell death by regulating the

condensation of chromatin.(102,103) The activation of protein kinases is frequently induced by cells treated with chemicals, supplements triggering apoptosis or growth factors. (104) Besides, serine/threonine kinase 4 plays a role in the immune system. It inhibits antiviral defence for example. STK4 deficiencies were analysed to impair T-cells and B-cells.(105-108) According to Nehme et al. STK4 mutations and the following effects of low expression levels showed immundeficiencies, autoimmunitiy and reduction of CD4- and CD8-T-cells in patients.(109) Furthermore the expression of the genes BCL2, IL7R and FOXO1 were downregulated in T cells of the patients, while the expression levels of FAS were increased. The downregulation of STK4 and FOXO1 was associated with apoptosis of naive T-cells.(109) Studies did show that polymorphism of MST1 correlates with colitis and Crohn's disease.(110,111) Several molecular pathways were shown to be affected by STK4, which depicted the importance of this target gene in cancer therapies.(112) STK4 lends itself as diagnostic biomarker for inflammation-induced hepatocellular carcinoma.(113) The expression of this multifunctional gene was also found to respond to L-carnitine levels, just like the other three genes.(2,91)

2. Materials and Methods

The following trials were designed to be performed in cells of a human hepatocellular carcinoma cell line and human primary skeletal muscle cells. All cells intended for L-carnitine supplementation were precultivated in our laboratory for three days in a carnitine deficient cell culture medium containing 10% dialysed FCS, and then induced by addition of the desired L-carnitine amount for four hours.(3) This precultivation in a medium containing 10% dialysed FCS guaranteed that the cells were all in a L-carnitine deficient condition, prior to subsequent supplementation with the metabolite.

2.1. Cell Culture Conditions

WRL68 = human epithelial heteroploid liver cell line:

-“Normal” WRL68 cells cultivated on medium with undialysed, normal 10% FCS

- 0µM-l-carnitine: WRL68 cells cultivated on medium with dialysed 10% FCS

- 40µM-L-carnitine: WRL68 cells cultivated on medium with dialysed 10% FCS and 40µM-L-carnitine induced.

- 80µM-L-carnitine: WRL68 cells cultivated on medium with dialysed FCS and 80µM-L-carnitine induced.

SKMC = human primary skeletal muscle cells

-“Normal” SKMC cultivated on medium with undialysed, normal 10% FCS

- 0µM-l-carnitine: SKMC cells cultivated on medium with dialysed 10% FCS

- 40µM-L-carnitine: SKMC cells cultivated on medium with dialysed 10% FCS and 40µM-L-carnitine induced.

- 80µM-L-carnitine: SKMC cells cultivated on medium with dialysed FCS and 80µM-L-carnitine induced.

2.1.1. WRL68

The WRL68 cell line is a human epithelial heteroploid liver cell line which represents liver characteristics. WRL68 cells are a good hepatic model system for investigations of complex liver functions. The function and morphology retains the same as in hepatocytes. The efficacy of some specific liver enzymes lasts.(114)

2.1.2. SKMC

The SKMC are human skeletal muscle cells, isolated from “normal” donors, according to the producer (Lonza Group). The cells are derived from gestational tissue, preferred from the psoas or quadriceps muscles.

2.2. cDNA Synthesis

To transfer RNA in cDNA the GoScript™ Reverse Transcription System of PROMEGA was prepared. The reaction kit of Promega was used, according to the usage information of the manufactorer. For the cDNA synthesis the cultivated RNA of the SKMC and the WRL68 cell line were applied. The concentration measurements were conducted with Thermo Scientific™ NanoDrop™ 2000.

Component	Volume, µl
GoScript™ 5X Reaction Buffer	4
MgCl2	3
PCR Nucleotide Mix	1
Recombinant RNasin® Ribonuclease Inhibitor	0,5
GoScript™ Reverse Transcriptase	1
Nuclease-Free Water to a final volume of 15µl	0,5
Total reaction mix volume	10,0

Table 1: Reaction Mix for cDNA Synthesis

5µl of the RNA was added to the reverse transcription reaction mix.

2.3. Primer

The primer selection was based on a literature research and a desired product size. The product size of the primers lies between a range of 200-300 bp. All primers were pretested to perform at the optimal annealing temperature. Therefore a Gradient PCR was performed, followed by a gel-electrophoresis.

Primer:

BCOX1
Sense: 5‘– TCT TCA CTC GGG GGA CTC AG -3‘
Antisense: 5‘– TGT GCG ATT GCT ATG CCG TT -3‘

TBD100
Sense: 5‘– CTG GCC AAG GTT GAA ACT CCA T -3‘
Antisense: 5‘– ACA CTG CAG TAT TAA AGA GAC GAA -3‘

LMNB1 1
Sense: 5‘- CCG TTC CTC TAA ACG CCA GC -3‘
Antisense: 5‘- CTG CGC ACC TTG TCG ATG TA -3‘

STK4 1
Sense: 5‘- GAA AGC GGA AGT GTG GGA GG -3‘
Antisense: 5‘- TAC GCT GCC ATA GGA CCC TT -3‘

2.4. Gradient PCR

For the pretest of the primers following reaction mix was prepared:

Component	Volume, µl
GoTaq® G2 Colorless Master Mix Promega	12,5
Upstream primer, 10µM	2,5
Downstream primer, 10µM	2,5
DNA template	1,0
Nuclease-free H2O	5,5
Total reaction mix volume	24,0µl

Table 2: Reaction Mix for Gradient PCR

The Mastercycler ® was used and programmed like in the table below.

Gradient PCR-Settings:

Step	°C	Duration
Initial denaturation	95	2 minutes
35 cycles:		
Denaturation	95	45 seconds
Annealing	61 (temperature increment of -1°C)	30 seconds
Elongation	72	45 seconds
Final elongation	72	2 minutes
Cooling of samples	8	-

Table 3: Temperature Setting for Gradient PCR

The gradient was set at 61°C and 8 x 0,2ml tubes were used. The column below shows the temperature range:

Tube row	1	2	3	4	5	6	7	8
Temperature	52,5	54,2	56,5	59,1	61,8	64,5	67,0	69,1

Table 4: Temperature Range for Gradient PCR

2.5. Gel-Electrophoresis

For analysis of the gradient PCR the GeneRuler™ 1kb DNA Ladder of Thermo Scientific™ was used. In a 1,2% agarose gel a mixture of 4,8µl 6XDNA loading Dye and 24µl reaction mix was loaded. Well-defined bands could be identified by using the DNA ladder as marker. The instruction was followed by using the manufacturer's user information. The DNA concentration was determined spectrophotometrically with the Thermo Scientific™ NanoDrop™ 2000 device.
The results showed the optimal annealing temperature at 61°degrees for two of four genes. While there were significant bands identified for TBDN100 and BCOX1, there were no meaningful results for LMNB1 and STK4 at any temperature. As a result of this, the data and their interpretation advised to redesign the primers.

New Primers:

LMNB1 2
Sense: 5'- GCG GTG TAC ATC GAC AAG GT -3'
Antisense: 5'- TCT CGA AGC TTG ATC TGG GC -3'

STK4 2
Sense: 5'- AAG GGA TGA GAC CAT GCA GC -3'
Antisense: 5'- TTG GAC TGG TAC TTC TGC CG -3'

2.6. Gel Elution and DNA Isolation

Following the instructions of the Wizard® SV Gel and PCR Clean-Up System the PCR-gel was eluted for a standard. For each target gene a serial dilution of the eluted DNA was generated, to calculate the amplification efficiency. As a result of this a standard curve was created.

2.7. Real Time PCR

All experiments were performed with the PCR detection system of BioRad (CFX96 Touch™ Real Time PCR Detection System). As intercalating fluorescent dye, SYBRGreen was selected. The following reaction mix, iTaq™ Universal SYBR® Green Supermix, was prepared as recommended by the manufactorer.:

Component	Volume, µl
iTaqUniversalSYBRGreenSupermix	10
Forward primer	0,5
Reverse primer	0,5
DNA template	1
Nuclease-free H2O	8
Total reaction mix volume	20µl

Table 5: Reaction Mix for Real Time PCR

The reaction mix was filled up on a white 96 well plate, 20µl per well. All reactions were replicated at least three times.

The table below shows the temperature protocol, which was chosen for the measurements.

Step	°C	Duration
Initial denaturation	95	2:00 minutes
Quantification 40 cycles	95	0:15 minute
Annealing temperature	61	0:15
	72	0:40

Table 6: Temperature Setting for Real Time PCR

2.8. Statistical Analysis

For the analysis of the real-time data, relative quantification was used as model. The Ct values of the target gene as well as of the reference gene are subtracted from the Ct values of the control. Afterwards the efficiency of the target gene to the power of ΔCt target is divided by the efficiency of the reference gene to the power of ΔCt reference.(115) The housekeeping gene ß-Actin is the reference gene in this study. The differences ΔCt of the values are used for simple t-test and serve as basis for ΔΔCt.(115) A minimum of three independent analyses were performed with a final t-test that revealed the statistical evidence about the accuracy of the triplicates. A significant result is a p-value less than 0,05%. The legend below describes the levels of significance.

Legend

- \- no results
- ° no significant values x >0,05
- * 0,01 > x <0,05
- ** 0,005 > x <0,01
- *** < 0,005

The statistical analysis was performed with the programs IBM SSPS Statistics 25 and the CFX Maestro™ Software from BioRad.

The Ct values and the efficiency of the reaction are calculated by the CFX Maestro™ Software. Ct is the quantification cycle determined by a single threshold line crossing the amplification curve. The threshold is set by the program at the point, where the fluorescence rises clearly over the amount of the template at the beginning of the measurement.(116)

The formula E=10^-1/slope defines the efficiency. The experiments were conducted with a serial dilution for reliability. A standard curve was created, which delivers the slope. The efficiency represents the effectiveness of the amplification, whereby doubling is the desirable amount per cycle and as such 2 is the ideal value. The tables below describe the efficiency rates of the genes in the different cell lines. STK4 shows the highest efficiency rate in both cell lines and TBDN100 the lowest. The performance of the amplification rates of the respective genes is similar in liver and skeletal muscle cells.

WRL68	
GENE	EFFICIENCY
LMNB1	1,727
BCOX1	1,609
STK4	2,013
TBDN100	1,637

Table 7: Efficiency of the amplification of the genes in the WRL68 liver cells.

SKMC	
GENE	EFFICIENCY
LMNB1	1,706
BCOX1	1,856
STK4	1,927
TBDN100	1,625

Table 8: Efficiency of the amplification of the genes in the SKMC cells.

R^2 is the coefficient of determination. It describes the deviation of the replicates from the standard curve or how well the desirable outcome is described by the replicates. The coefficient should lie between 0,98 and 1. In this study all measurements have a coefficient of determination higher than 0,98 and lower than 1.

3. Results

The analysis of the steady state mRNA levels of the analysed genes showed significant differences between expression levels of the target sequences. The mRNA levels were measured in a comparative manner in SKMC and WRL68 liver cells (muscle versus liver cells). All investigations showed statistically significant results in the WRL68 liver cells. In the SKMC some results diverge.

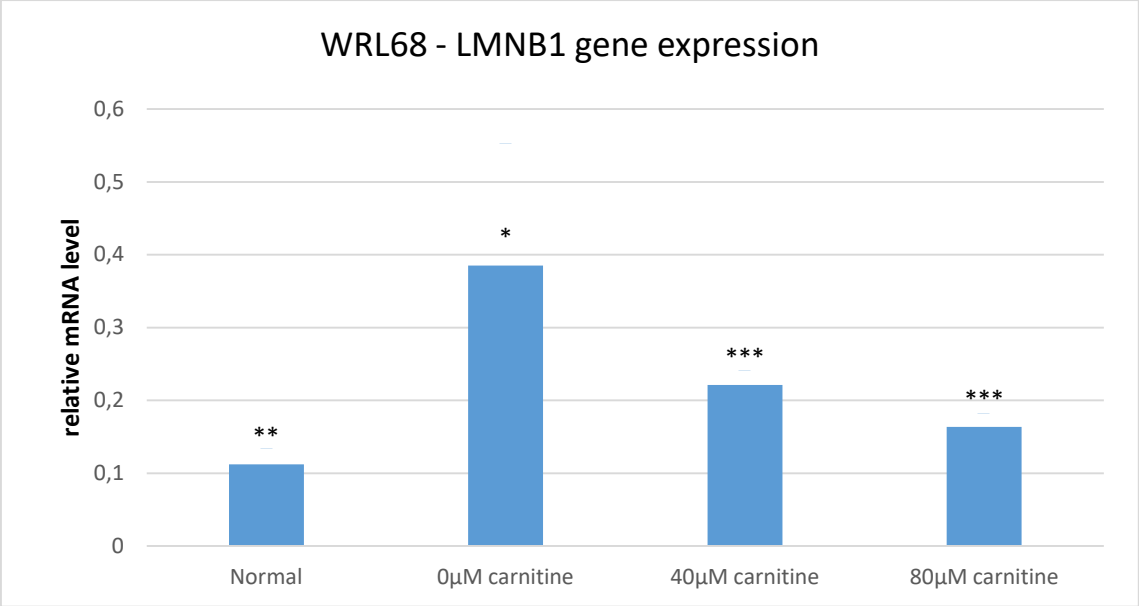

Figure 1: Relative mRNA levels of LaminB1 in the WRL68 liver cell line grown in 10% FCS under various conditions.

The 40µM and 80µM samples have similar significance levels and both show increased levels compared to the sample “Normal”. The highest relative mRNA levels are represented in the dialysed sample.

The calculation of the relative quantification of LMNB1 in the SKMC could not be achieved. The variation range of the data was too high.

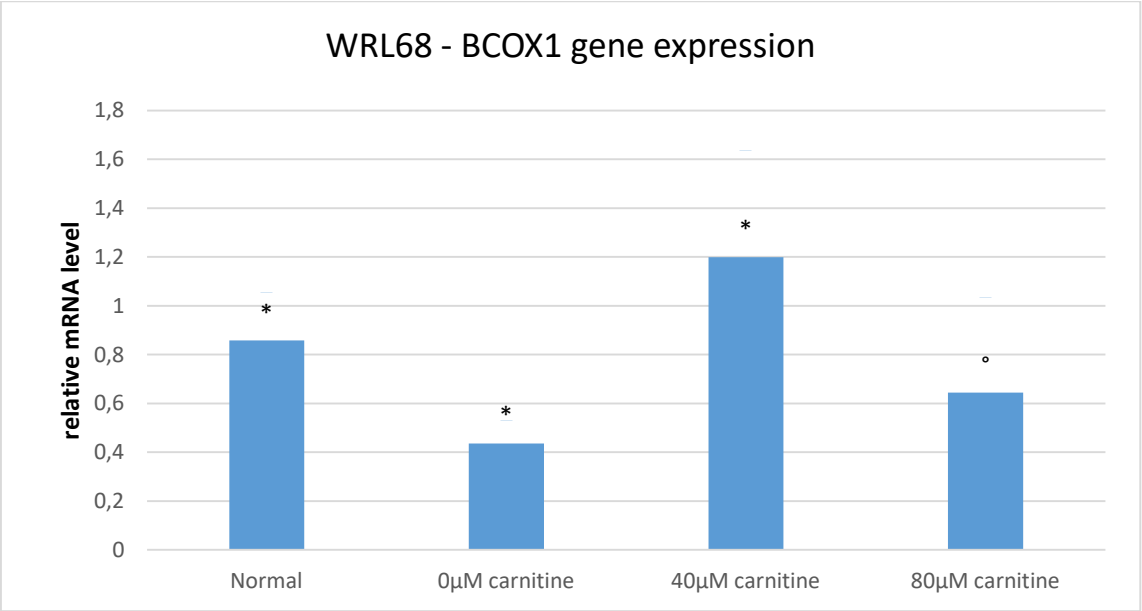

Figure 2: Relative mRNA levels of BCOX1 in the WRL68 liver cell line grown in 10% FCS under various conditions.

All results had similar statistical significance levels (<0, 05 using t-test) except of the 80µM sample. (p=0,104) 40µM L-carnitine supplementation as cultivation condition has the highest value.

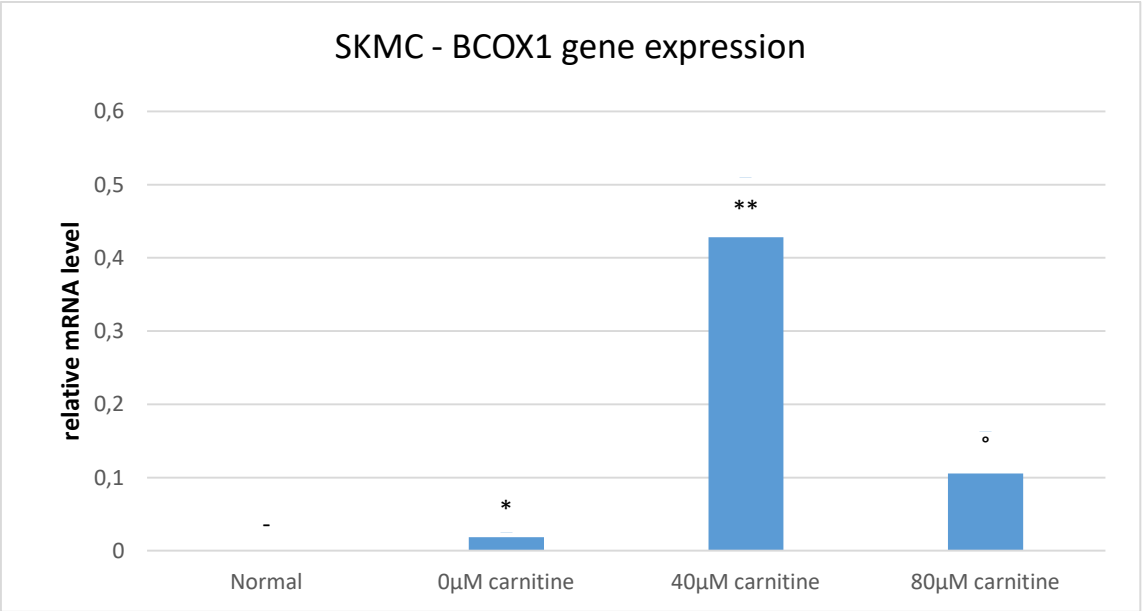

Figure 3: Relative mRNA levels of BCOX1 in the SKMC grown in 10% FCS under various conditions.

The calculation of the relative mRNA level of the sample “Normal” of BCOX1 in the SKMC could not be achieved. The variation range of the data was too high. The value of the 80µM sample was statistically not significant. p=0,086 The relative mRNA level of 40µM cultivation condition is obviously increased compared to the dialysed mRNA level.

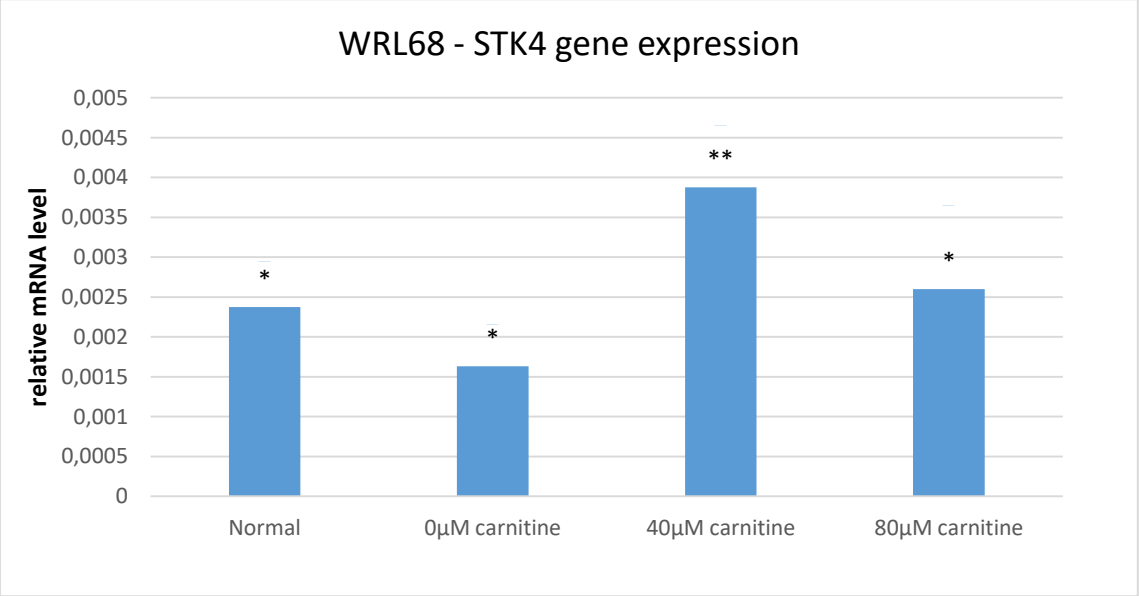

Figure 4: Relative mRNA levels of STK4 in the WRL68 liver cell line grown in 10% FCS under various conditions.

All results were statistically significant. 40µM L-carnitine supplementation as cultivation condition is increased compared to the other samples.

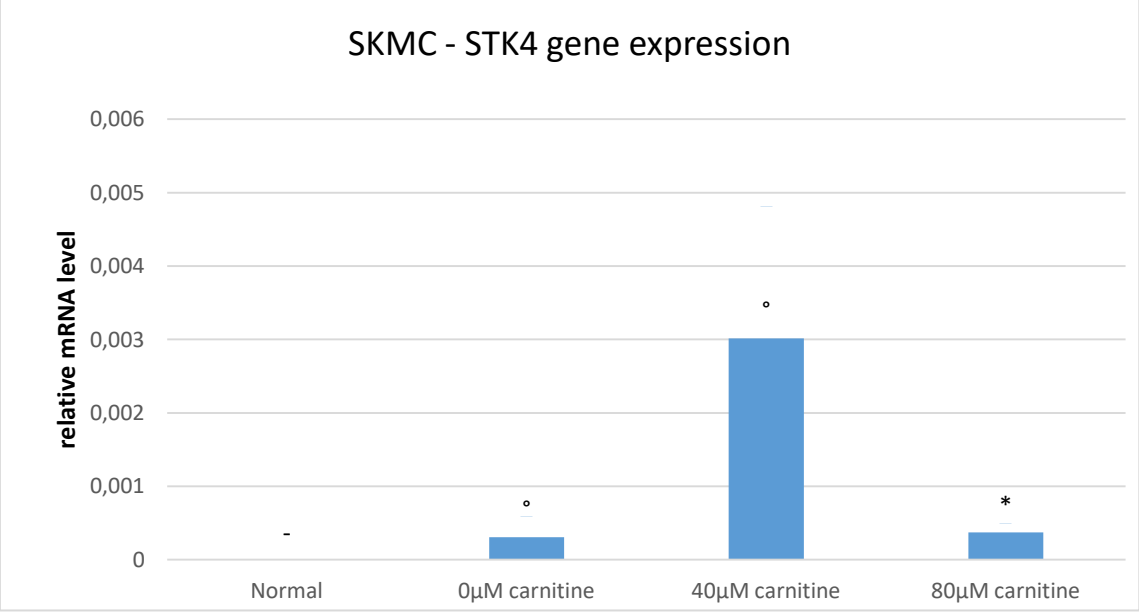

Figure 5: Relative mRNA levels of STK4 in the SKMC grown in 10% FCS under various conditions.

The STK4 gene expression depicts just one statistically significant result – 80µM-sample ($p<0,08$ using t-test), 40µM-sample (0,100),Dialysed ($p=0,202$). The results of the physiological cultivation condition “Normal” doesn’t allow any reliable prediction due the available data.

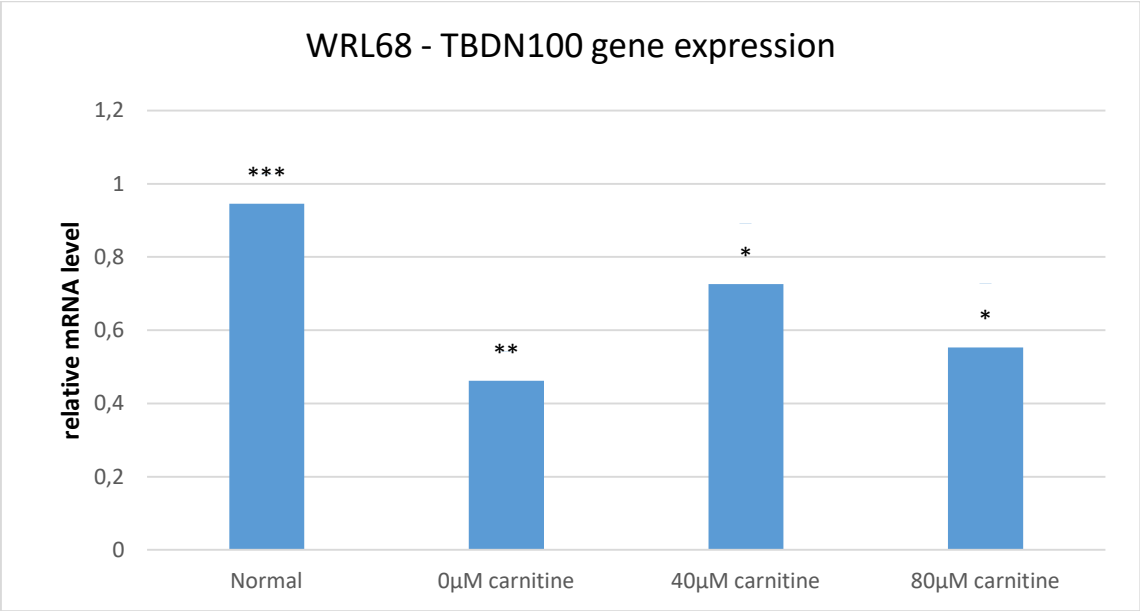

Figure 6: Relative mRNA levels of TBDN100 in the WRL68 liver cell line grown in 10% FCS under various conditions.

All results were statistically significant. Especially the sample “Normal” showed high significance and a high relative mRNA level.

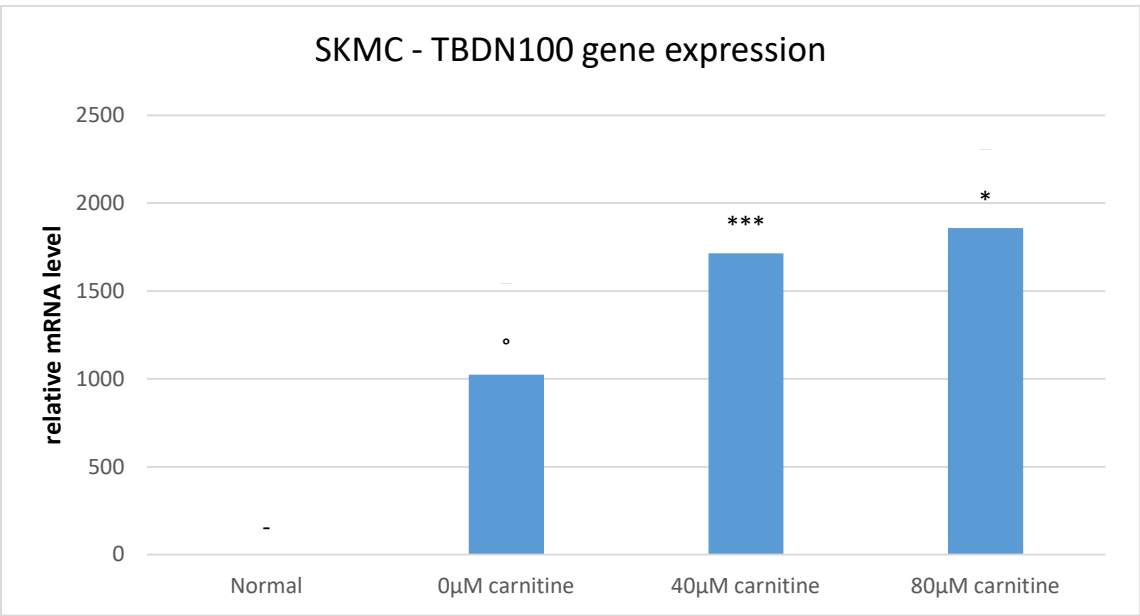

Figure 7: Relative mRNA levels of TBDN100 in the SKMC grown in 10% FCS under various conditions.

The 40µM - L-carnitine supplemented sample showed a statistically high significance level. (p=<0,005 using t-test) The dialysed sample has a significance level of p=0,077. The results of the physiological cultivation condition “Normal” doesn’t allow any reliable prediction due the available data.

Further results are based on normalised relative quantification by using the ΔΔCt - method. ß-Actin still serves as housekeeping gene for normalising the expression levels. The ΔΔCt – method is based on the assumption that there is no difference in the amount of the starting material or the efficiency of the gene amplification.(117) Normalisation reduces the variance of the expression levels.(118) To approach an efficiency calibrated model the corrected efficiencies were implicated in the calculation.(115,116,118,119)

The condition described as “Normal” represents cell lines grown in standard growth medium and served as a control for the individual analysis of the target genes in the two cell lines. The different cultivation conditions, „0µM“ L-carnitine, „ 40µM“ L-carnitine, „80µM“ L-carnitine described cultivation in growth medium supplemented with dialysed 10% fetal calf serum and the given L-carnitine levels. All these mRNA samples were compared in relation to the normal untreated physiological sample. The investigated genes were LMNB1, BCOX1, STK4 and TBDN100. To be able to draw any conclusions from the differences of relative normalised expression levels between the WRL68 and SKMC the qPCR results of the investigated genes were put into perspective.

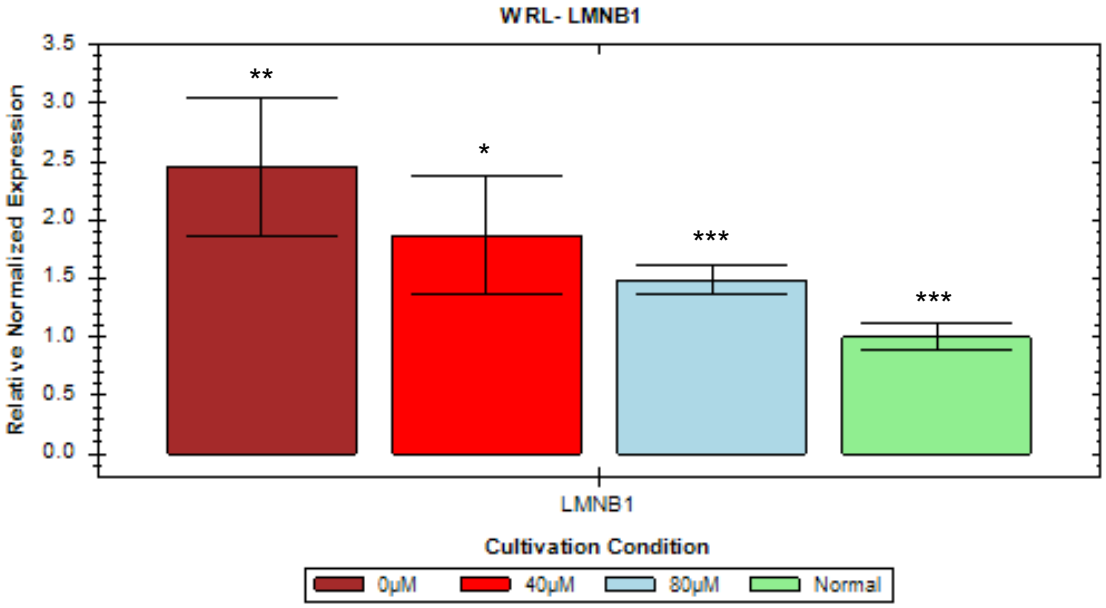

Figure1: The relative normalised expression levels of LaminB1 in the WRL68 liver cell line grown in 10% FCS under various conditions.
The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The relative normalised expression levels of Lamin B1 in the liver cells showed a descending trend from an induced level correlated to the amount of L-carnitine supplementation compared to the normal growth condition. ($p<0,01$ using t-test) The normal physiological state of gene expression in the liver cell, was set at 1,00 and served as an expression control. WRL68 cells grown in dialysed 10% FCS exhibited 2,5-fold induction ($p<0,05$ using t-test) of LMNB-1 steady state mRNA levels compared to normal growth conditions with undialysed 10% FCS. 40µM L-carnitine supplementation as cultivation condition in dialysed 10% FCS nearly reached an expression level of 2 (1,86 $p<0,05$ using t-test) compared to the normal condition level. 80µM L-carnitine supplementation in dialysed 10% FCS as cultivation condition performs a half time higher expression level (1,49) of LaminB1 than in 10% FCS. ($p<0,05$ using t-test)

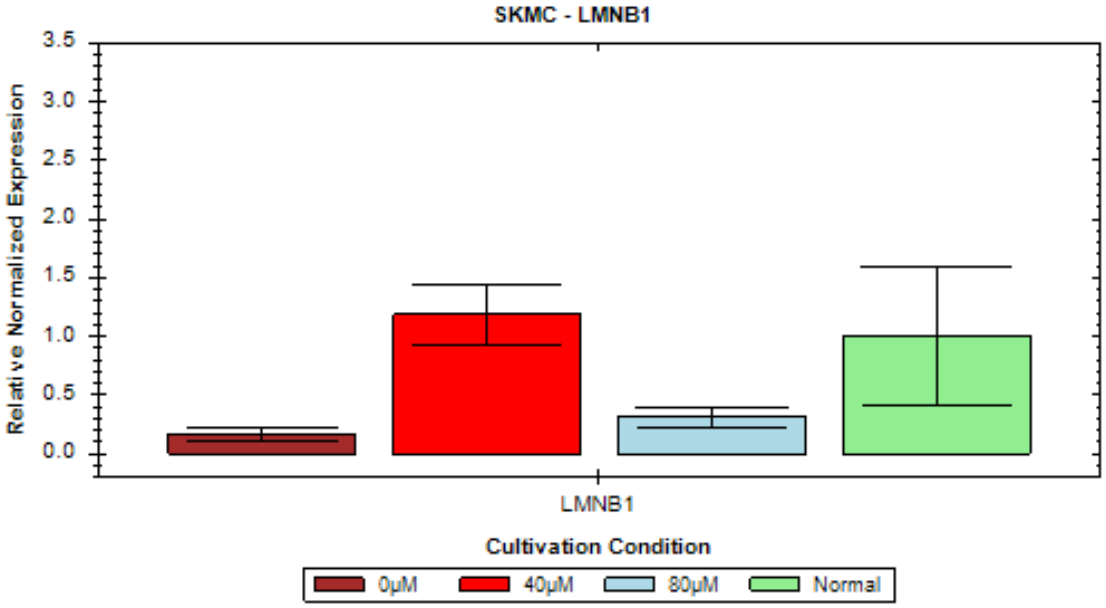

Figure 8: The relative normalised expression levels of LaminB1 in skeletal muscle cells grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The analysis of LMNB1 expression in the SKMC showed no statistical significant results under all cultivation conditions. The figure shows the relative normalised expression levels of LMNB1 of the experiment. The normal physiological state of gene expression in the skeletal muscle cells, is valued as 1,00 and serves as sample control. LaminB1 as target gene is in all samples underexpressed compared to the control except of the 40µM sample. The gene expression of LaminB1 in the skeletal muscle cells grown under condition with no L-carnitine (0µM - SKMC grown in dialysed 10% FCS) showed levels just above zero (0,17). 80µM – cultivation condition could not reinduce expression levels up to normal (0,31). However 40µM L-carnitine supplementation as cultivation condition was able to superinduce LMNB1 steady state expression levels even higher compared to normal (1,25).

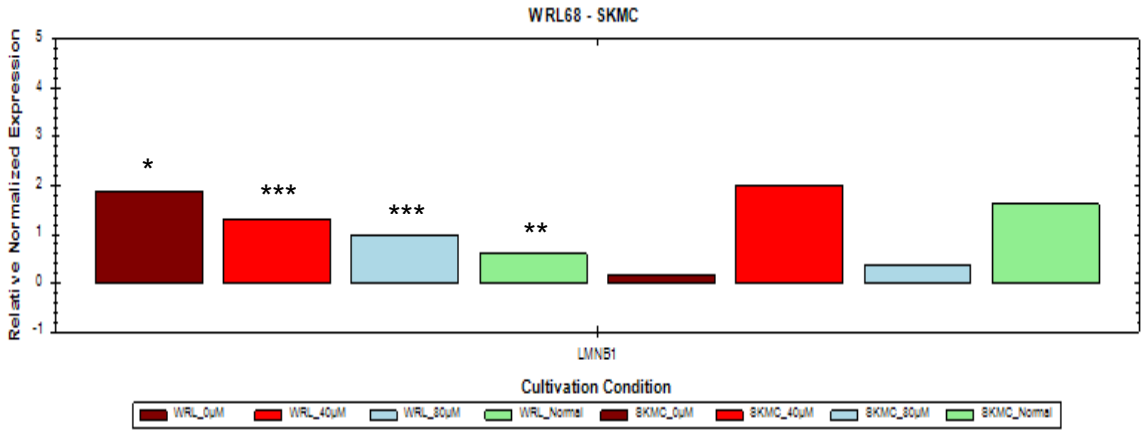

Figure 9: The relative normalised expression levels of LMNB1 in comparison between skeletal muscle cells and WRL68 liver cells.

The comparison of mRNA steady state levels of LMNB1 in SKMC and WRL68 liver cells cultivated under various growth conditions. (“ 0µM”, “40µM” and “80µM” L-carnitine)

WRL68 and skeletal muscle cells exhibit marked differences in expression levels of the LMNB1 gene compared to the physiological state. While the expression levels in the liver cells slightly increased, the outcome in the skeletal muscle cells has mostly a decreasing tendency, except a very interesting peak at 40µM L-carnitine supplementation level. The 0µM sample (dialysed FCS only) showed the highest amount of specific PCR products in the liver cells, in the skeletal muscle cells the lowest.

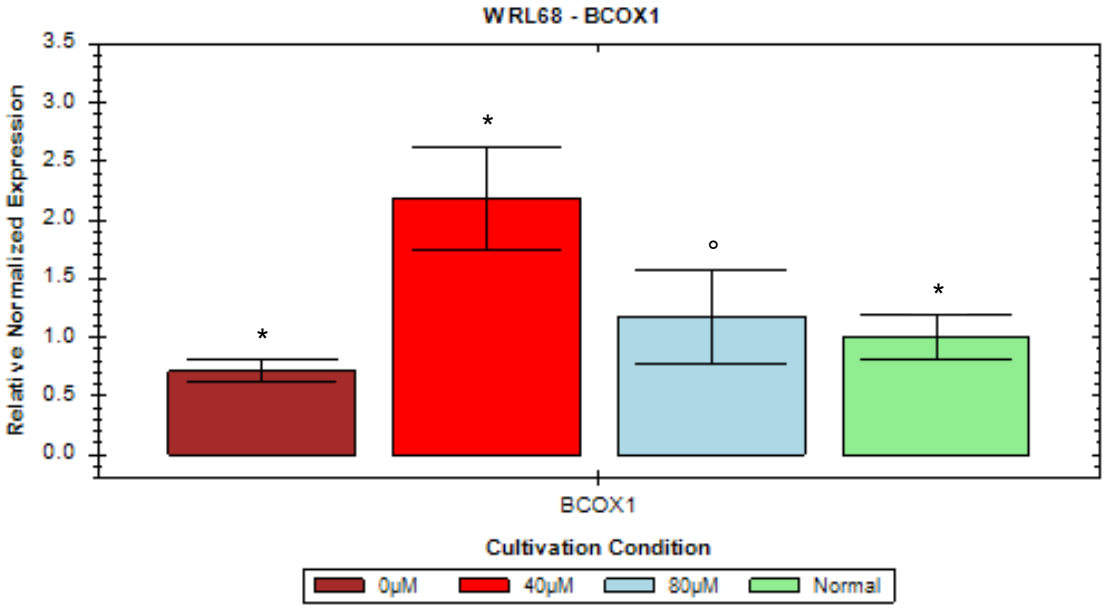

Figure 10: The relative normalised expression levels of BCOX1 in the WRL68 liver cell line grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of BCOX1 in liver cells, was valued as 1,00 and served as untreated control. The cultivation condition of 0µM L-carnitine provided a relative mRNA level of 0,71 compared to the normal condition. ($p<0,05$ using t-test) High levels of BCOX1 expression were detected in the liver cells cultivated under 40µM L-carntine supplementation. With a value of 2,19 the gene expression of BCOX1 under cultivation condition 40µM, had more than doubled. ($p<0,05$ using t-test) This means that 40 µM L-carnitine was able to super-induce the transcript level significantly. The BCOX1 expression levels (1,18) of the liver cells, cultivated under dialysed 10% FCS supplemented with 80µM L-carnitine, was slightly higher compared to the control, but characterised by low statistical significance.

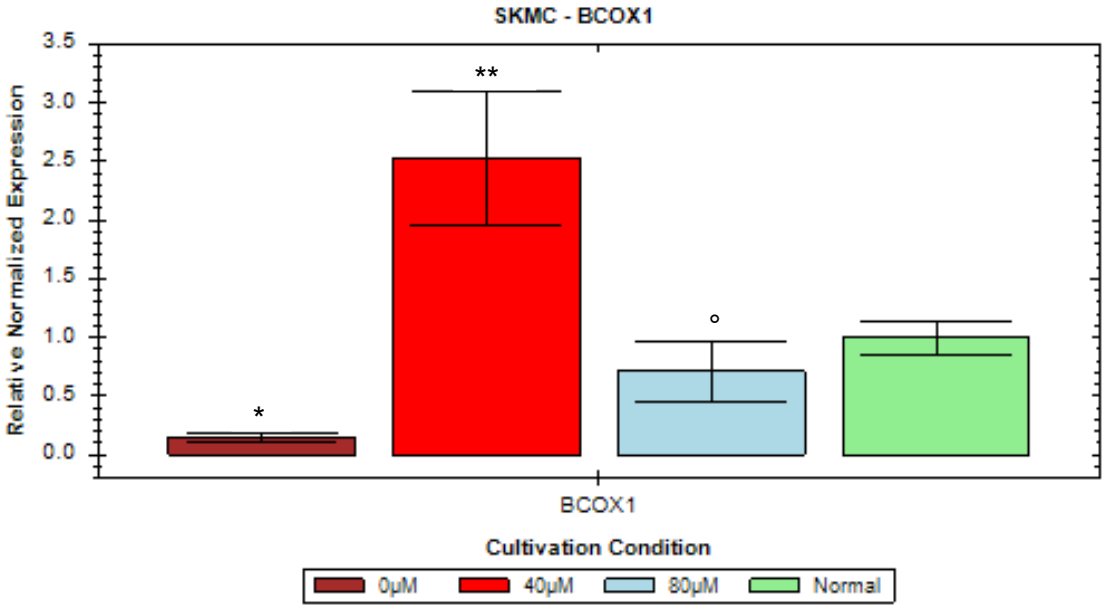

Figure 11: The relative normalised expression levels of BCOX1 in skeletal muscle cells grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of BCOX1 in the skeletal muscle cells, was set at 1,00 and served as an untreated control. While the samples 0µM ($p<0,05$ using t-test) and 80µM showed a marked decrease in the expression level of the target gene BCOX1 compared to the control, the sample isolated from cells grown with 40µM L-carnitine exhibited a strong induction (overexpression) ($p<0,01$ using t-test). The fold in- or reduction of the analysed samples of the skeletal muscle cells cultivated under different L-carnitine supplementation levels were: 0µM - 0,14, 40µM - 2,53, 80µM - 0,71. It has to be noted, that the results of the physiological cultivation condition “Normal” do not allow any reliable prediction due the normalisation process based on this condition.

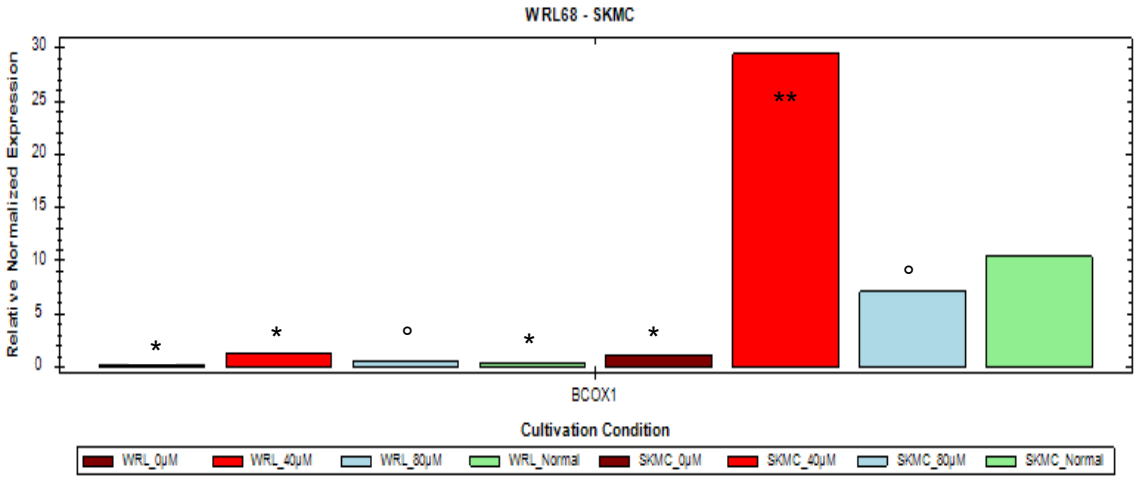

Figure 12: The relative normalised expression levels of BCOX1 in comparison of skeletal muscle cells and WRL68 liver cells.

The comparison of mRNA steady state levels of LMNB1 in SKMC and WRL68 liver cells cultivated under various growth conditions. (“0µM”, “40µM” and “80µM” L-carnitine)

The mRNA samples isolated from 40µM L-carnitine supplementation gave rise to the highest steady state BCOX1 mRNA levels in liver cells as well as in skeletal muscle cells. ($p<0,05$ using t-test) Both exhibited a more than doubled fold induction compared to the untreated, normal physiological state of growth. Anyway the expression level of BCOX1 under the 40µM condition is noticeably high in the SKMC. In the liver cells the relative normalised expression level of BCOX1 in the 80µM sample was slightly increased compared to normal conditions, in the skeletal muscle cells slightly decreased. Both cell lines cultivated in dialysed 10% FCS markedly repressed BCOX1 mRNA steady state levels, whereby the values in the skeletal muscle cells were much lower. ($p<0,05$ using t-test)

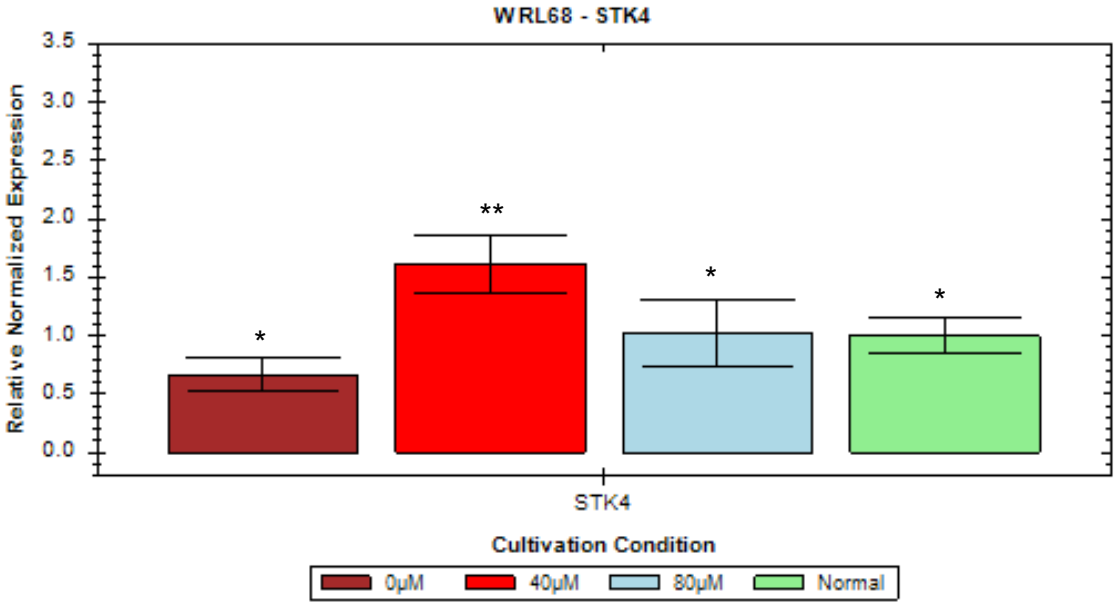

Figure 13: The relative normalised expression levels of STK4 in the WRL68 liver cell line grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of the serine/threonine kinase 4 in the liver cells, was set at 1,00 and served as normal, untreated control. STK4 as target gene exhibited an up- and downregulation collinear with the L-carnitine levels in the investigated WRL68-cells. The cultivation condition of 40µM L-carnitine supplementation shows a gene expression value of 1,61. ($p<0,01$ using t-test) The relative normalised expression level of STK4 is in the 80µM sample (1,02) almost the same as in the normal control. ($p<0,05$ using t-test) WRL68 liver cells grown in dialysed 10% FCS downregulate STK4 steady state mRNA levels about 30 % (0,67). ($p<0,05$ using t-test)

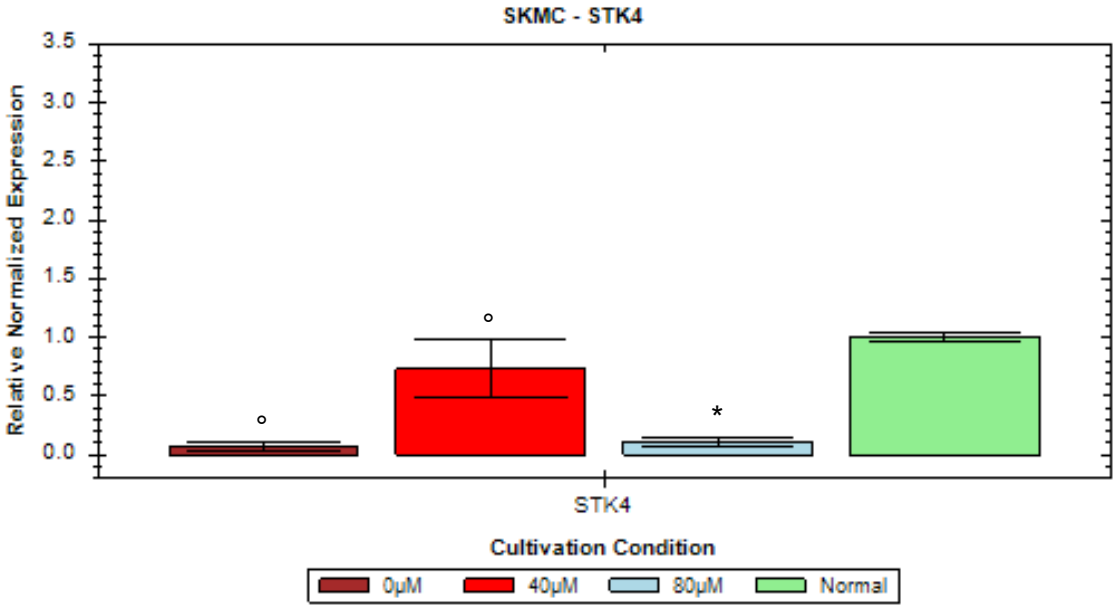

Figure 14: The relative normalised expression levels of STK4 in skeletal muscle cells grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of the serine/threonine kinase 4 in skeletal muscle cells, was set at 1,00 and served as normal, untreated control. The gene expression levels of STK4 reveal in 0 and 80 µM L-carnitine a marked downregulation of the STK4 gene, only in the case of 40 µM L-carnitine a distinct re-induction closer to normal physiological levels could be observed. The gene expression of the serine/threonine kinase in the skeletal muscle cells grown under condition with no L-carnitine (0µM - SKMC grown in dialysed 10% FCS) showed levels just above zero (0,07). 80µM cultivation condition gave rise to similar low transcript amounts of 0,11 compared to the normal target expression level in skeletal muscle cells. ($p<0,05$ using t-test) However, the 40µM L-carnitine supplementation level during cultivation showed the highest gene expression level (0,73), being not far beyond the normal physiological condition. It has to be noted, that the results of the physiological cultivation condition “Normal” don’t allow any reliable prediction due to the normalisation process based on this condition.

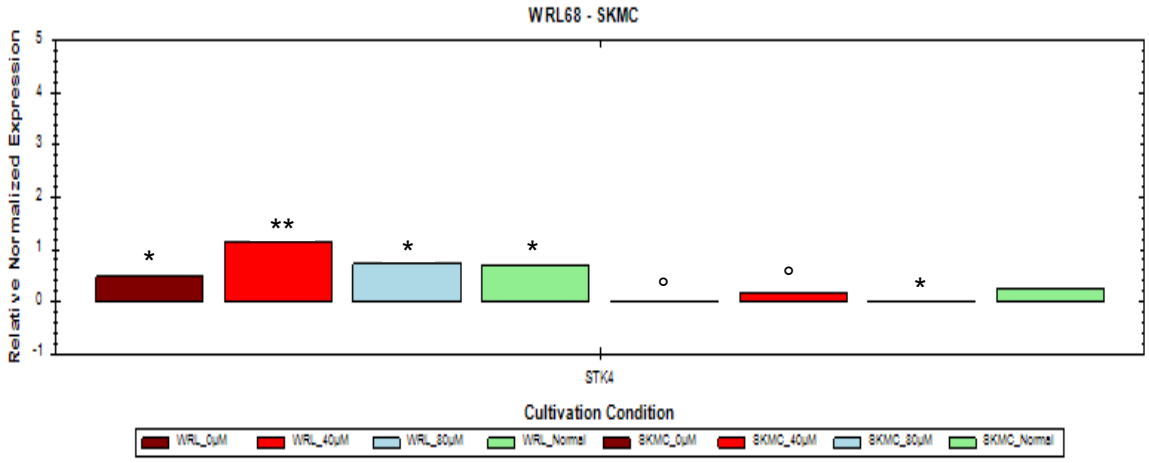

Figure 15: The relative normalised expression levels of STK4 in comparison of skeletal muscle cells and WRL68 liver cells.

The comparison of mRNA steady state levels of LMNB1 in SKMC and WRL68 liver cells cultivated under various growth conditions. (“0µM”, “40µM” and “80µM” L-carnitine)

As depicted in figure 9 the normalised expression levels of STK4 in both analysed cell lines are presented adjacent to each other. In the skeletal muscle cells the expression levels of the serine/threonine kinase 4 was more pronounced downregulated in all samples as in WRL68 cells. In liver cells the L-carnitine dialysed cultivated sample (0µM) had lower levels compared to physiological treatment. ($p<0,05$ using t-test) The values of the 40µM sample reached mRNA steady state levels over 1,5-fold in the WRL68 cells and 0,73-fold in the SKMC cells compared to the control. ($p<0,01$ using t-test) This makes it clear that at this L-carnitine level the strongest inductional effect could be observed in the liver cells. The figure has similarity to figure 3 where the steady state mRNA expression levels of LMNB1 are presented.

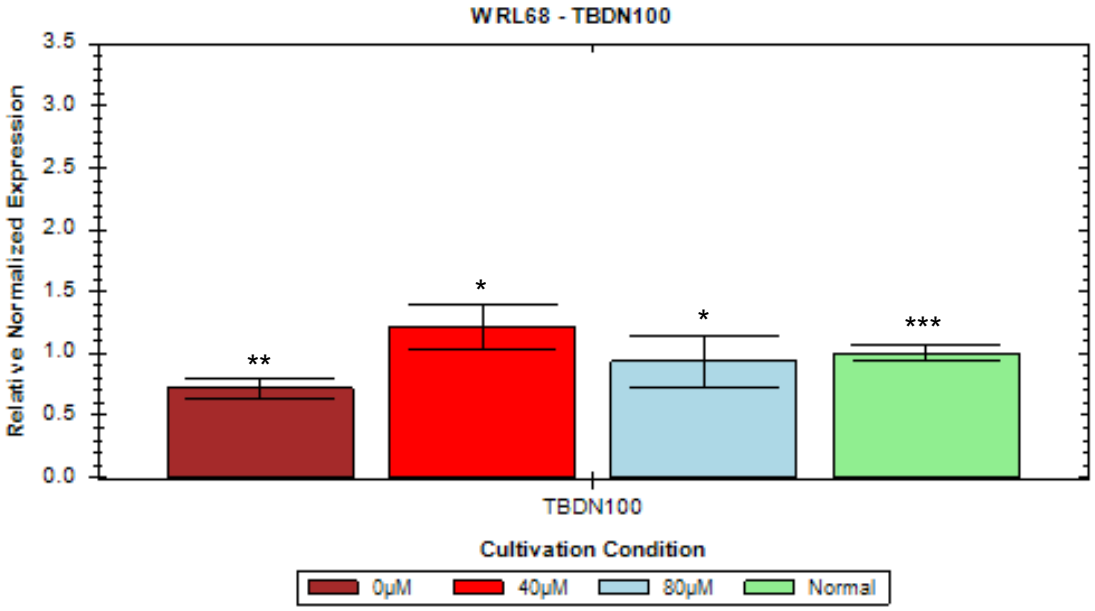

Figure 16: The relative normalised expression levels of TBDN100 in the WRL68 liver cell line grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of TBDN100 in the WRL68 liver cell line, was set at 1,00 and served as normal, untreated control. The relative normalised expression levels of the 0µM sample was 0,72. ($p<0,01$ using t-test) The mRNA steady state levels of the 40µM sample was a 1,21 fold induction of the normal condition sample. ($p<0,05$ using t-test) The relative normalised expression levels of TBDN100 in the WRL68 liver cell line grown in 10% FCS under 80µM supplementation of L-carnitine was 0,94. ($p<0,05$ using t-test). Also in the case of the TBDN100 gene 40 µM L-carnitine was able to superinduce the transcript levels above normal.

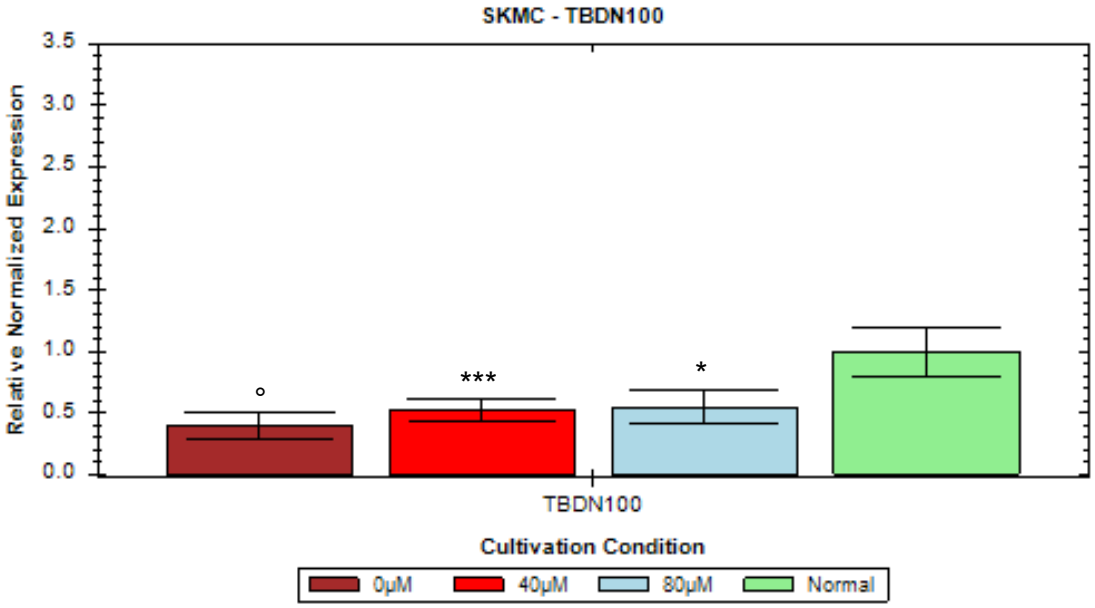

Figure 17: The relative normalised expression levels of TBDN100 in skeletal muscle cells grown in 10% FCS under various conditions.

The control „Normal“ was set at 1,00 and was compared to the other conditions „0µM“ (dialysed 10%FCS), „40µM“ (dialysed 10%FCS supplemented) and „80µM“ (dialysed 10%FCS supplemented). All additives to the growth medium lasted for exactly four hours.

The normal physiological state of gene expression of TBDN100 in skeletal muscle cells, was set at 1,00 and served as normal, untreated control. The relative normalised expression levels of TBDN100 in skeletal muscle cells show a downregulation in all samples. The values for the cultivation condition samples were: 0µM - 0,39, 40µM - 0,52 ($p<0,005$ using t-test), 80µM - 0,55 ($p<0,05$ using t-test). It has to be noted, that the results of the physiological cultivation condition “Normal” don’t allow any reliable prediction due to the normalisation process based on this condition.

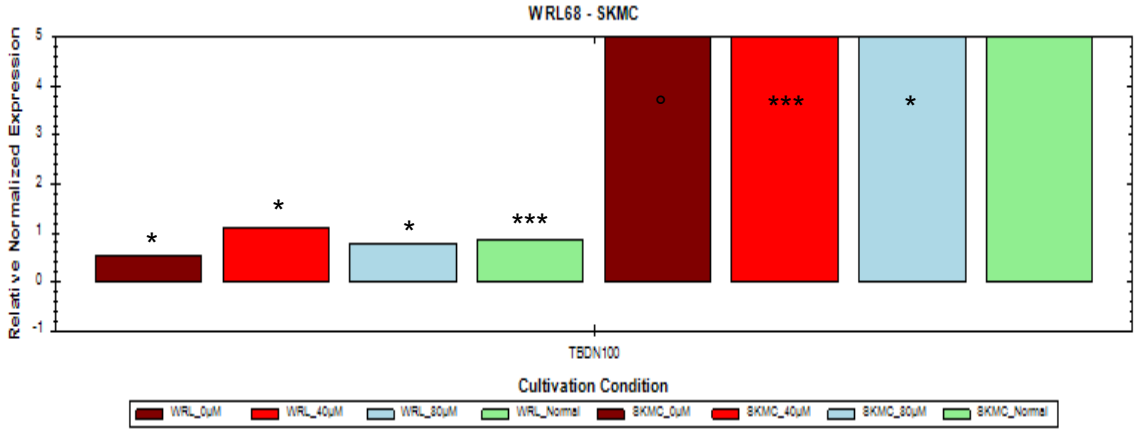

Figure 18: The relative normalised expression levels ofTBDN100 in comparison of skeletal muscle cells and WRL68 liver cells, focused on the WRL68 cell line.

The comparison of mRNA steady state levels of LMNB1 in SKMC and WRL68 liver cells cultivated under various growth conditions. (“0µM”, “40µM” and “80µM” L-carnitine)

From figure 12 it is apparent that there is a low mRNA steady state level of TBDN100 in all four different cultivated liver cells. In comparison to that, the gene expression in SKMC is high.

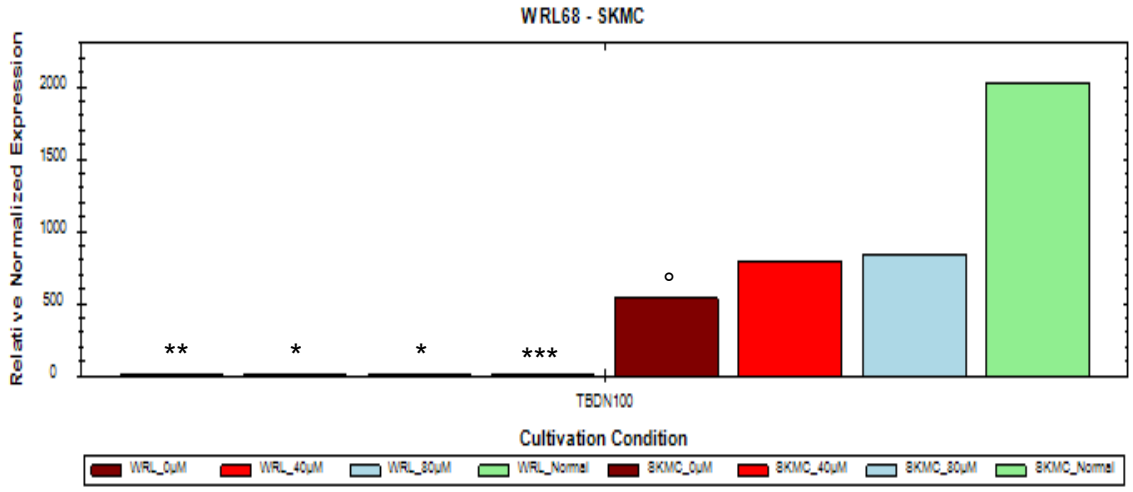

Figure 19: The relative normalised expression levels of TBDN100 in comparison of skeletal muscle cells and WRL68 liver cells, focused on the SKMC.

The comparison of mRNA steady state levels of LMNB1 in SKMC and WRL68 liver cells cultivated under various growth conditions. (“0µM”, “40µM” and “80µM” L-carnitine)

As mentioned before there is a clearly defined difference between the expression levels of TBDN100 in WRL68 liver cells and SKMC. All analysed conditions in liver cells showed an enormous under-expression of the target gene compared to the physiological condition of L-carnitine in the SKMC. In the skeletal muscle cells the highest TBDN100 transcript level could be defined in the normal control. while all L-carnitine supplementation steps showed lower levels . In contrast, in the liver cells a slight under-expression occurred in the 0µM and the 80µM cell batch.($p<0,05$ using t-test) Only under the 40µM L-carnitine level in WRL68 liver cells higher expression levels of TBDN100 than in liver cells grown under physiological conditions could be found. ($p<0,05$ using t-test) It is worth noting that the results of the physiological cultivation condition “Normal” don’t allow any reliable prediction due to the normalisation process based on this condition.

4. Discussion

L-carnitine is a nutrigenomic factor and as such it plays a key role in the human metabolism. The background of this study is to examine the effects of various L-carnitine supplementations on gene expression. Therefore, four genes of a previously performed microarray analysis were selected, which had responded well to L-carnitine influence.(1) The four genes and their functions in the human body were well described above. This research addresses a comparison between simulated physiological conditions and modified conditions of the culture medium for the cells by measuring amplification rates of the genes after cultivation. Further, the question whether there are differences between the two different cell types, which would be logical according their function for the whole organism, or not, is the key issue of this research.

The two chosen cell types, representing the liver and skeletal muscles, differ enormously in their functions. The pivotal function of the liver is the preservation of the energy supply in the organism. For that it metabolises the provided nutrients. In absence of nutrients, the liver catabolises macromolecules for gluconeogenesis. Further on it biosynthesises proteins and stabilises the acid base balance. It is the only organ in a human beings body, which produces ketone bodies. This feature is important for the energy supply of the brain. In the skeletal muscles, glycogen is simply reserved for energy supply and has a constant level. In the liver cell it is the threshold for stabilising the blood glucose level and differs enormously. Cell division is absent in mature muscle cells.

Several studies point to the fact that L-carnitine is highly concentrated and stored in muscle cells.(5-7) Furthermore skeletal muscles are presumed as place of synthesis of 6N-trimethyl-L-lysine. This indicates the importance of the association of muscle tissue and L-carnitine in the human body. In contrast to that, the liver has a pivotal impact on the biosynthesis of L-carnitine, even when the amount of synthesised L-carnitine is small.(5) According to Rebouche 6N-trimethyl-L-lysine dioxygenase is found in mitochondria of tissues like liver, kidney, heart, skeletal muscles, and brain.(5) All of this points to the fact that different concentration levels of L-carnitine in liver and muscle cells might influence the metabolism of humans in general as well as the gene expression of the genes detected in the microarray analysis in particular.(2,91)

A microarray analysis had revealed that L-carnitine is a regulating nutrient in gene expression of hundreds of genes.(2,91) Out of this huge number four genes have been selected for the investigation based on their presumed pivotal impact on the genomics of important cell and tissue specific body functions, as described earlier. Values of those genes based on the microarray analysis and the actual results are listed below. WRL68 cells have been supplemented with 80 µM L-carnitine and show influences on gene functions.

Dia 80 vs Dia 0 fold change			
Gene	**Microarray analysis**	**qPCR results** (actual study)	
		WRL68	SKMC
TBDN100	5,24	1,20	1,82
STK4	3,86	1,59	1,21
BCOX1	3,14	1,48	5,65
LMNB1	2,45	0,42	-

Table 9: Comparison between the microarray analysis and the actual results. (1) The fold change between supplementation with 0µM and 80µM L-carnitine

The data would seem to suggest further investigations by L-carnitine supplementation. In this study the gene expression was compared under four different cultivation conditions: 0µM, 40µM, 80µM and the “Normal” physiological condition. All simulated cultivation conditions were compared to the normal state.

The results revealed deviations from the data of the microarray analysis. There is much less difference between the values of the relative expression levels of TBDN100 in the 0µM and the 80µM sample. The 80µM sample still demonstrates the increase of gene expression under L-carnitine induction, but not the fivefold of the 0µM sample. The other results of the genes showed similar performances.

WRL68				
Gene	**0µM carnitine**	**40µM carnitine**	**80µM carnitine**	**Normal**
TBDN100	0,46	0,73	0,55	0,95
STK4	0,00	0,00	0,00	0,00
BCOX1	0,44	1,20	0,64	0,86
LMNB1	0,39	0,22	0,16	0,11

Table 10: Results of the relative quantification of the four genes and the supplementation levels in the WRL68 liver cells.

SKMC				
Gene	**0µM carnitine**	**40µM carnitine**	**80µM carnitine**	**Normal**
TBDN100	1023,71	1715,30	1859,28	n.a.
STK4	0,00	0,00	0,00	n.a.
BCOX1	0,02	0,43	0,11	n.a.
LMNB1	n.a.	n.a.	n.a.	n.a.

Table 11: Results of the relative quantification of the four genes and the supplementation levels in the SKMC cells.

This study compared the WRL68 and SKMC cell lines and revealed a lot of differences between liver and skeletal muscle. It tended to much more variation in the gene expression levels of SKMC, as can be seen in table 8 and 9, the steady state mRNA level of the gene STK4 led to the relatively same low mRNA steady state levels in muscle cells and liver cells under all cultivation conditions. The analysis of LMNB1 expression in the SKMC resulted in induction levels especially with 40 µM L-carnitine even above normal. But it has to be mentioned that in this case measurements did not reach statistical significance in all cultivation conditions. In the liver cells all values were statistically significant, with the highest induction level at 0 µM L-carnitine (under dialysed FCS only).

WRL68 and skeletal muscle cells exhibited considerable differences in expression levels of the LMNB1 gene compared to the physiological state. LaminB1 basically is a structure protein. The increased gene expression levels of LMNB1 in the liver cells caused by L-carnitine supplementation could be explained by the increased production of amino acids in this tissue. L-carnitine directly supports and enhances the mitochondrial function and energy production. Remarkably, WRL68 cells grown in dialysed 10% FCS showed the highest LMNB-1 steady state mRNA

levels. One can conclude from that, that L-carnitine variation has much influence on the essential functions of LaminB1 as a regulating protein. It regulates chromosome functions or structural and morphological properties in liver and skeletal muscle cells.(92-94) In the capacity of these functions, variable LaminB1 expression levels have impact on the preservation of the nuclear envelope in male reproductive cells after meiosis.(97) Duplication of this gene led to increased expression levels of Lamin B1 in lymphoblasts and was associated with autosomal dominant leukodystrophy (ADLD).(98,99) The latter being a disease of the central nervous system generated by a loss of myelin. Overexpression of this protein in cultured cells showed an altered nuclear morphology.(98) Additionally it is to mention, that LMNB1 serves as biomarker for early stages of a hepatocellular carcinoma. (100) There is a relationship between steatosis and a reduced function of ß-oxidation and therefore with L-carnitine as well.(43,44) In absence of L-carnitine, the long chain fatty acids could not be transported into the mitochondria for energy supply. One hypothesis is that L-carnitine deficiency not only leads to steatosis as a possible early stage of hepatocellular carcinoma, the overexpression of LMNB1 might benefit ADLD as well.

The serine/threonine kinase 4 is similarly important like as the previously discussed genes, because it influences cell proliferation as well as cell death.(101-103) But related to the other genes, the steady state mRNA levels of STK4 had the lowest values. All supplementation levels showed higher amplification rates in the WRL68 cells compared to the SKMC. As mentioned above the specific functions of the liver might be the reason for that. While protein proliferation takes place in the liver, the storage of proteins occurs in the skeletal muscles. STK4 deficiency was detected to affect the immune defence. (109) The liver produces enzymes and proteins for the immune defence. The downregulation of STK4 and FOXO1 was associated with apoptosis of naive T-cells.(109) L-carnitine is known for its antioxidant, anti-inflammatory and anticytokine features.(23) STK4 lends itself as diagnostic biomarker for inflammation-induced hepatocellular carcinoma.(113)

The values of BCOX1 and TBDN100 showed significant differences between SKMC and the WRL68 liver cell line. ($p<0,05$ using t-test) While the steady state

mRNA levels of BCOX1 in the liver cells showed fewer differences between the cultivation conditions, BCOX1 expression in SKMC differed enormously. The 40µM sample revealed a significant high value. ($p<0,05$ using t-test) The other three samples showed much less expression levels of BCOX1, whereby the values of the 0µM and the 80µM sample dropped beyond the physiological state. An explanation for this might be the fact, that 40µM L-carnitine supplementation as cultivation condition is the closest to the physiological state in adults. The gene BCOX 1 was overexpressed in breast and prostate carcinoma. L-carnitine is highly contained in red meat. There was a correlation established between red meat and an increase of breast and prostate carcinoma.(120) In the liver are several proteins and fatty acids produced which are related to cancer. (121) Assumptions were made that insulin growth hormone was linked to prostate carcinoma as well.(122) This growth factor is produced in liver cells. This might indicate a correlation between these facts and a BCOX1 increase in the liver cells. Further on, an abnormal L-carnitine uptake by nutrition might suggest a higher biosynthesis of proteins in general and proteins related to cancer in special. The skeletal muscle cells are the reservoir for L-carnitine. L-carnitine improves energy supply by encouraging the transport of long chain fatty acids. Fat is an important source of energy for carcinoma. One can conclude from these facts that high L-carnitine supplementation led to the high expression levels of BCOX1. But the results suggested that L-carnitine supplementation in the concentration range of 40µM has the highest expression level of BCOX1 in skeletal muscle and liver cells.

The expression levels of TBDN100 showed the highest differences between WRL68 cells and SKMC. The results might be achieved by the differences between the functions of the tissues. In the liver cells were almost no changes detectable and the steady state transcript level was low. The TBDN100 mRNA level of the physiological sample in the SKMC reached a high state. This might be a result of L-carnitine inducing muscle growth and therefore protein proliferation was needed. Supplementation of L-carnitine as cultivation condition lowered the outcome more than half, whereby 10% dialysed FCS as cultivation condition led to the lowest mRNA level. TBDN100 is a part of the N-alpha-acetyltranferase complex.(83-85) This complex catalyses the process of protein modification after translation.(85) Impairment of this complex might lead to apoptosis.(84) As a result

of this, TBDN100 gained more importance in tumour cells.(83) There was a correlation detected between downregulation of NATH and increased age in several tissues, like nervous system and heart as well.(90) This might be a reasonable explanation for the downregulation compared to the normal state. Adults skeletal muscle cells are fully differentiated and are no longer divisible. All three L-carnitine modified samples, 0µM, 40µM ($p<0,05$ using t-test) and 80µM ($p<0,05$ using t-test) showed a downregulation in the gene expression levels, though the lowest values were generated in the 0µM sample. The apparent data regarding this gene, indicate that L-carnitine supplementation lowers the gene expression of TBDN100 in SKMC. Acetylation of histones plays a key role in transcriptional dysregulation which might be an explanation for downregulation of TBDN100 in SKMC as well.(86)

A limiting factor in this study was the sample size, although all measurements were performed at least in triplicates. The statistical significance would increase with an enlargement of the sample size. In addition, the low concentration levels might also be questionable due to the dynamical range of the standard curve. Nevertheless all investigations showed significant results in the WRL68 liver cells. In the SKMC some results diverged. Further on the efficiencies of the qPCR of three genes (LMNB1, BCOX1, STK4) were less than the desired value (90%<>110%) for a qualitative research. This thesis works to the first time compared relevant genes between two important organ systems regarding L-carnitine usage and function and in a logical way it opened a door for further investigations.

Examining other cell types like kidney cells might be interesting for continuing works. Kidney cells are also places of synthesis of L-carnitine.(5) Other options might be a variation of the amount of L-carnitine supplementation or examination of additional interesting genes. Researches in our laboratory showed also interesting results in cell cultivation with L-carnitine supplementation under hyperlipidemic and hyperglycemic conditions.(3,4) That leads to diseases like diabetes mellitus or hyperlipidemia. Another modifiable factor very likely is duration of L-carnitine induction. Within this thesis we mainly focused on immediate effects by allowing only four hours of L-carnitine induction on immediate effects, affecting

transcription and promoter factors. Longer lasting increased L-carnitine levels for 24 or 48 hours would provide additional interesting information.

5. Conclusion

In conclusion, this study the first time delivered organ specific differences in gene expression after L-carnitine deprivation and subsequent supplementation. In parallel further experimental evidences of L-carnitine and its effects on gene expression were raised. Taking into account the statistical data, all examined genes showed significant influence of L-carnitine on the amplification rate. The most noteworthy result was the difference between the gene expression of TBDN100 in the WRL68 liver cells and the SKMC. Besides, the gene expression of TBDN100 showed downregulation correlated to the amount of L-carnitine induction. Another important aspect is the significant positive correlation between BCOX1 and the 40µM L-carnitine supplementation as cultivation condition. Especially the gene expression levels of BCOX1 of the 40µM sample of the SKMC showed increased values. All in all, further investigations will be necessary to illuminate the organ specific transcriptional effects of the nutrigenomic factor L-carnitine.

6. References

1. Hofer-Litzlbauer, E. (2005) *Biochemical and genetical consequences of carnitine deficiency caused by downregulation of the carnitine acyltransferase genes.*Doctoral Thesis, Medical University of Vienna
2. Hofer-Litzlbauer, E., Molzer, S., Kienesberger, K., Bilban, M., Wagner, O., and Hofbauer, R. (in submission) Microarray analysis of carnitine deficiency and supplementation reveals that the human genome is heavily influence on transcriptional level.
3. Indra, D. (2018) *Analysis of the impact of L-carnitine as a nutrigenomic factor on various nuclear receptor pathways.* Master of Science, University of Vienna
4. Panzenböck, A. (2017) *Effects of L-Carnitine on Gene Expression Under Hyperlipidemic and Hyperglycemic Conditions.* Dr.med.univ., Medical University of Vienna
5. Rebouche, C. J. (1992) Carnitine function and requirements during the life cycle. *The FASEB Journal* **6**, 3379-3386
6. Fujisawa, K., Takami, T., Matsuzaki, A., Matsumoto, T., Yamamoto, N., Terai, S., and Sakaida, I. (2017) Evaluation of the effects of L-carnitine on medaka (Oryzias latipes) fatty liver. *Scientific Reports* **7**, 2749
7. El-Hattab, A. W., and Scaglia, F. (2015) Disorders of carnitine biosynthesis and transport. *Molecular genetics and metabolism* **116**, 107-112
8. WANDERS, R. J. (2002) Carnitine biosynthesis in mammals. *Biochemical Journal* **361**, 417-429
9. Strijbis, K., Vaz, F. M., and Distel, B. (2010) Enzymology of the carnitine biosynthesis pathway. *IUBMB life* **62**, 357-362
10. McGarry, J. D., and Brown, N. F. (1997) The mitochondrial carnitine palmitoyltransferase system—from concept to molecular analysis. *European Journal of Biochemistry* **244**, 1-14
11. Lee Carter, A., Abney, T. O., and Lapp, D. F. (1995) Biosynthesis and metabolism of carnitine. *Journal of Child Neurology* **10**, 2S3-2S7
12. Bremer, J. (1983) Carnitine--metabolism and functions. *Physiological reviews* **63**, 1420-1480
13. Adeva-Andany, M. M., Calvo-Castro, I., Fernández-Fernández, C., Donapetry-García, C., and Pedre-Piñeiro, A. M. (2017) Significance of l-carnitine for human health. *IUBMB life* **69**, 578-594
14. Kuhajda, F. P., and Ronnett, G. V. (2007) Modulation of carnitine palmitoyltransferase-1 for the treatment of obesity. *Current opinion in investigational drugs (London, England: 2000)* **8**, 312-317
15. Dambrova, M., and Liepinsh, E. (2015) Risks and benefits of carnitine supplementation in diabetes. *Experimental and Clinical Endocrinology & Diabetes* **123**, 95-100
16. Liepinsh, E., Makrecka, M., Kuka, J., Cirule, H., Makarova, E., Sevostjanovs, E., Grinberga, S., Vilskersts, R., Lola, D., and Loza, E. (2014) Selective inhibition of OCTN2 is more effective than inhibition of gamma-butyrobetaine dioxygenase to decrease the availability of l-carnitine and to reduce myocardial infarct size. *Pharmacological research* **85**, 33-38
17. Muoio, D. M., Noland, R. C., Kovalik, J.-P., Seiler, S. E., Davies, M. N., DeBalsi, K. L., Ilkayeva, O. R., Stevens, R. D., Kheterpal, I., and Zhang, J. (2012) Muscle-specific deletion of carnitine acetyltransferase compromises glucose tolerance and metabolic flexibility. *Cell metabolism* **15**, 764-777

18. Evans, A. (2003) Dialysis-related carnitine disorder and levocarnitine pharmacology. *American journal of kidney diseases* **41**, S13-S26
19. Hedayati, S. S. (2006) HEMATOLOGY: ISSUES IN THE DIALYSIS PATIENT: Dialysis-Related Carnitine Disorder. in *Seminars in dialysis*, Wiley Online Library
20. Wang, Y., Ye, J., Ganapathy, V., and Longo, N. (1999) Mutations in the organic cation/carnitine transporter OCTN2 in primary carnitine deficiency. *Proceedings of the National Academy of Sciences* **96**, 2356-2360
21. Karlic, H., Lohninger, A., Laschan, C., Lapin, A., Böhmer, F., Huemer, M., Guthann, E., Rappold, E., and Pfeilstöcker, M. (2003) Downregulation of carnitine acyltransferases and organic cation transporter OCTN2 in mononuclear cells in healthy elderly and patients with myelodysplastic syndromes. *Journal of molecular medicine* **81**, 435-442
22. Fathi, E., and Farahzadi, R. (2014) Application of L-carnitine as nutritional supplement in veterinary medicine. *Rom J Biochem* **51**, 31-41
23. Aydin, A., Halici, Z., Albayrak, A., Polat, B., Karakus, E., Yildirim, O. S., Bayir, Y., Cadirci, E., Ayan, A. K., and Aksakal, A. M. (2015) Treatment with carnitine enhances bone fracture healing under osteoporotic and/or inflammatory conditions. *Basic & clinical pharmacology & toxicology* **117**, 173-179
24. O'Connor, J., Costell, M., Miguez, M., Portolés, M., and Grisolía, S. (1987) Effect of L-carnitine on ketone bodies, redox state and free amino acids in the liver of hyperammonemic mice. *Biochemical pharmacology* **36**, 3169-3173
25. Pochini, L., Scalise, M., Galluccio, M., and Indiveri, C. (2013) OCTN cation transporters in health and disease: role as drug targets and assay development. *Journal of biomolecular screening* **18**, 851-867
26. Farahzadi, R., Fathi, E., Mesbah-Namin, S. A., and Zarghami, N. (2018) Anti-aging protective effect of L-carnitine as clinical agent in regenerative medicine through increasing telomerase activity and change in the hTERT promoter CpG island methylation status of adipose tissue-derived mesenchymal stem cells. *Tissue and Cell* **54**, 105-113
27. Longo, N., Frigeni, M., and Pasquali, M. (2016) Carnitine transport and fatty acid oxidation. *Biochimica et Biophysica Acta (BBA)-Molecular Cell Research* **1863**, 2422-2435
28. Marquis, N. R., and Fritz, I. B. (1965) The distribution of carnitine, acetylcarnitine, and carnitine acetyltransferase in rat tissues. *Journal of Biological Chemistry* **240**, 2193-2196
29. Arsenian, M. A. (1997) Carnitine and its derivatives in cardiovascular disease. *Progress in cardiovascular diseases* **40**, 265-286
30. Brass, E. P., Hoppel, C. L., and Hiatt, W. R. (1994) Effect of intravenous L-carnitine on carnitine homeostasis and fuel metabolism during exercise in humans. *Clinical Pharmacology & Therapeutics* **55**, 681-692
31. Brass, E. P., and Hiatt, W. R. (1994) Carnitine metabolism during exercise. *Life sciences* **54**, 1383-1393
32. Imbe, A., Tanimoto, K., Inaba, Y., Sakai, S., Shishikura, K., Imbe, H., Tanimoto, Y., Terasaki, J., Imagawa, A., and Hanafusa, T. (2018) Effects of L-carnitine supplementation on the quality of life in diabetic patients with muscle cramps. *Endocrine journal* **65**, 521-526
33. Wang, Z.-Y., Liu, Y.-Y., Liu, G.-H., Lu, H.-B., and Mao, C.-Y. (2018) l-Carnitine and heart disease. *Life sciences* **194**, 88-97

34. DiNicolantonio, J. J., Lavie, C. J., Fares, H., Menezes, A. R., and O'keefe, J. H. (2013) L-carnitine in the secondary prevention of cardiovascular disease: systematic review and meta-analysis. in *Mayo Clinic Proceedings*, Elsevier
35. Rasmussen, J., Nielsen, O. W., Lund, A. M., Køber, L., and Djurhuus, H. (2013) Primary carnitine deficiency and pivalic acid exposure causing encephalopathy and fatal cardiac events. *Journal of inherited metabolic disease* **36**, 35-41
36. Karaca, K., and Solmaz, F. (2017) The Effect of Carnitine on Peripheral Nerve Regeneration. *Journal of Craniofacial Surgery* **28**, e160-e165
37. Pescosolido, N., Pascarella, A., Nebbioso, M., and Scarsella, G. (2014) Degenerative events in retina and optic nerve induced by inhibition of carnitine transport. *Journal of biological regulators and homeostatic agents* **28**, 263-270
38. Sakurauchi, Y., Matsumoto, Y., Shinzato, T., Takai, I., Nakamura, Y., Sato, M., Nakai, S., Miwa, M., Morita, H., Miwa, T., Amano, I., and Maeda, K. (1998) Effects of L-carnitine supplementation on muscular symptoms in hemodialyzed patients. *Am J Kidney Dis* **32**, 258-264
39. Nakanishi, H., Kurosaki, M., Tsuchiya, K., Nakakuki, N., Takada, H., Matsuda, S., Gondo, K., Asano, Y., Hattori, N., and Tamaki, N. (2015) L-carnitine reduces muscle cramps in patients with cirrhosis. *Clinical Gastroenterology and Hepatology* **13**, 1540-1543
40. Cleak, M., and Eston, R. (1992) Muscle soreness, swelling, stiffness and strength loss after intense eccentric exercise. *British journal of sports medicine* **26**, 267-272
41. Fielding, R., Riede, L., Lugo, J., and Bellamine, A. (2018) l-Carnitine supplementation in recovery after exercise. *Nutrients* **10**, 349
42. Abdelrazik, H., and Agarwal, A. (2009) L-carnitine and assisted reproduction. *Archives of Medical Science Special Issues* **2009**, 47-47
43. Korenaga, M., Wang, T., Li, Y., Showalter, L. A., Chan, T., Sun, J., and Weinman, S. A. (2005) Hepatitis C virus core protein inhibits mitochondrial electron transport and increases reactive oxygen species (ROS) production. *The Journal of biological chemistry* **280**, 37481-37488
44. Tsukuda, Y., Suda, G., Tsunematsu, S., Ito, J., Sato, F., Terashita, K., Nakai, M., Sho, T., Maehara, O., Shimazaki, T., Kimura, M., Morikawa, K., Natsuizaka, M., Ogawa, K., Ohnishi, S., Chuma, M., and Sakamoto, N. (2017) Anti-adipogenic and antiviral effects of l-carnitine on hepatitis C virus infection. *Journal of medical virology* **89**, 857-866
45. Dunning, K. R., and Robker, R. L. (2012) Promoting lipid utilization with l-carnitine to improve oocyte quality. *Animal reproduction science* **134**, 69-75
46. Agarwal, A., Sengupta, P., and Durairajanayagam, D. (2018) Role of L-carnitine in female infertility. *Reproductive Biology and Endocrinology* **16**, 5
47. Pfeilschifter, J., Köditz, R., Pfohl, M., and Schatz, H. (2002) Changes in proinflammatory cytokine activity after menopause. *Endocrine reviews* **23**, 90-119
48. Orsal, E., Halici, Z., Bayir, Y., Cadirci, E., Bilen, H., Ferah, I., Aydin, A., Ozkanlar, S., Ayan, A. K., and Seven, B. (2013) The role of carnitine on ovariectomy and inflammation-induced osteoporosis in rats. *Experimental Biology and Medicine* **238**, 1406-1412
49. Faloni, A., Sasso-Cerri, E., Katchburian, E., and Cerri, P. S. (2007) Decrease in the number and apoptosis of alveolar bone osteoclasts in estrogen-treated rats. *Journal of periodontal research* **42**, 193-201

50. Jansson, L., and Holmdahl, R. (1998) Estrogen-mediated immunosuppression in autoimmune diseases. *Inflammation research : official journal of the European Histamine Research Society ... [et al.]* **47**, 290-301
51. Da Silva, J., Larbre, J., Spector, T., Perry, L., Scott, D., and Willoughby, D. (1993) Protective effect of androgens against inflammation induced cartilage degradation in male rodents. *Annals of the rheumatic diseases* **52**, 285
52. Josefsson, E., Tarkowski, A., and Caristen, H. (1992) Anti-inflammatory properties of estrogen: I. In vivo suppression of leukocyte production in bone marrow and redistribution of peripheral blood neutrophils. *Cellular immunology* **142**, 67-78
53. Carlsten, H., Nilsson, N., Jonsson, R., Bäckman, K., Holmdahl, R., and Tarkowski, A. (1992) Estrogen accelerates immune complex glomerulonephritis but ameliorates T cell-mediated vasculitis and sialadenitis in autoimmune MRL lpr/lpr mice. *Cellular immunology* **144**, 190-202
54. Brass, E. P., and Hiatt, W. R. (1998) The role of carnitine and carnitine supplementation during exercise in man and in individuals with special needs. *Journal of the American College of Nutrition* **17**, 207-215
55. Chiu, K., Keller, E., Crenshaw, T., and Gravenstein, S. (1999) Carnitine and dehydroepiandrosterone sulfate induce protein synthesis in porcine primary osteoblast-like cells. *Calcified tissue international* **64**, 527-533
56. Malaguarnera, M., Cammalleri, L., Gargante, M. P., Vacante, M., Colonna, V., and Motta, M. (2007) l-Carnitine treatment reduces severity of physical and mental fatigue and increases cognitive functions in centenarians: a randomized and controlled clinical trial–. *The American journal of clinical nutrition* **86**, 1738-1744
57. Nazary-Vannani, A., Ghaedi, E., Mousavi, S. M., Teymouri, A., Rahmani, J., and Varkaneh, H. K. (2018) The effect of L-carnitine supplementation on serum leptin concentrations: a systematic review and meta-analysis of randomized controlled trials. *Endocrine* **60**, 386-394
58. Duranay, M., Akay, H., Yilmaz, F. M., Senes, M., Tekeli, N., and Yucel, D. (2006) Effects of L-carnitine infusions on inflammatory and nutritional markers in haemodialysis patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **21**, 3211-3214
59. Malaguarnera, M., Vacante, M., Bertino, G., Neri, S., Malaguarnera, M., Gargante, M. P., Motta, M., Lupo, L., Chisari, G., Bruno, C. M., Pennisi, G., and Bella, R. (2011) The supplementation of acetyl-L-carnitine decreases fatigue and increases quality of life in patients with hepatitis C treated with pegylated interferon-alpha 2b plus ribavirin. *Journal of interferon & cytokine research : the official journal of the International Society for Interferon and Cytokine Research* **31**, 653-659
60. Biolo, G., Stulle, M., Bianco, F., Mengozzi, G., Barazzoni, R., Vasile, A., Panzetta, G., and Guarnieri, G. (2008) Insulin action on glucose and protein metabolism during L-carnitine supplementation in maintenance haemodialysis patients. *Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association* **23**, 991-997
61. Mantovani, G., Maccio, A., Madeddu, C., Gramignano, G., Serpe, R., Massa, E., Dessi, M., Tanca, F. M., Sanna, E., Deiana, L., Panzone, F.,

Contu, P., and Floris, C. (2008) Randomized phase III clinical trial of five different arms of treatment for patients with cancer cachexia: interim results. *Nutrition (Burbank, Los Angeles County, Calif.)* **24**, 305-313
62. An, J. H., Kim, Y. J., Kim, K. J., Kim, S. H., Kim, N. H., Kim, H. Y., Kim, N. H., Choi, K. M., Baik, S. H., and Choi, D. S. (2016) L-carnitine supplementation for the management of fatigue in patients with hypothyroidism on levothyroxine treatment: a randomized, double-blind, placebo-controlled trial. *Endocrine journal* **63**, 885-895
63. Dambrova, M., Makretskaia, M., Vilshkersts, R., Kuka, I., and Liepin'sh, É. (2014) Carnitine as a marker of atherosclerosis and other risks of cardiovascular diseases. *Kardiologiia* **54**, 91-96
64. Koeth, R. A., Wang, Z., Levison, B. S., Buffa, J. A., Org, E., Sheehy, B. T., Britt, E. B., Fu, X., Wu, Y., and Li, L. (2013) Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine* **19**, 576
65. Nasca, C., Bigio, B., Lee, F. S., Young, S. P., Kautz, M. M., Albright, A., Beasley, J., Millington, D. S., Mathé, A. A., and Kocsis, J. H. (2018) Acetyl-l-carnitine deficiency in patients with major depressive disorder. *Proceedings of the National Academy of Sciences* **115**, 8627-8632
66. Godárová, A., Litzlbauer, E., Brunner, S., Agu, A. C., Lohninger, A., and Hofbauer, R. (2005) L-Carnitine Regulates mRNA Expression Levels of the Carnitine Acyltransferases–CPT1A, CPT2, and CRAT. *Monatshefte für Chemie/Chemical Monthly* **136**, 1349-1363
67. Arenas, J., Ricoy, J., Encinas, A., Pola, P., D'iddio, S., Zeviani, M., DiDonato, S., and Corsi, M. (1991) Carnitine in muscle, serum, and urine of nonprofessional athletes: effects of physical exercise, training, and l-carnitine administration. *Muscle & Nerve: Official Journal of the American Association of Electrodiagnostic Medicine* **14**, 598-604
68. Wang, Z., Klipfell, E., Bennett, B. J., Koeth, R., Levison, B. S., Dugar, B., Feldstein, A. E., Britt, E. B., Fu, X., Chung, Y. M., Wu, Y., Schauer, P., Smith, J. D., Allayee, H., Tang, W. H., DiDonato, J. A., Lusis, A. J., and Hazen, S. L. (2011) Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57-63
69. Stephens, F. B., Wall, B. T., Marimuthu, K., Shannon, C. E., Constantin-Teodosiu, D., Macdonald, I. A., and Greenhaff, P. L. (2013) Skeletal muscle carnitine loading increases energy expenditure, modulates fuel metabolism gene networks and prevents body fat accumulation in humans. *The Journal of physiology* **591**, 4655-4666
70. Vidal-Casariego, A., Burgos-Peláez, R., Martinez-Faedo, C., Calvo-Gracia, F., Valero-Zanuy, M., Luengo-Pérez, L., and Cuerda-Compés, C. (2013) Metabolic effects of L-carnitine on type 2 diabetes mellitus: systematic review and meta-analysis. *Experimental and clinical endocrinology & diabetes* **121**, 234-238
71. Rebouche, C. J. (1991) Quantitative estimation of absorption and degradation of a carnitine supplement by human adults. *Metabolism* **40**, 1305-1310
72. Pan, Y., and Lu, F. (2017) Supplementation with high-dose L-carnitine on hemodialysis tolerance in uremic patients with severe heart diseases. *Zhonghua yi xue za zhi* **97**, 3792-3795
73. Kraemer, W. J., Volek, J. S., Spiering, B. A., and Vingren, J. L. (2005) L-carnitine supplementation: a new paradigm for its role in exercise. *Monatshefte für Chemie/Chemical Monthly* **136**, 1383-1390

74. Hülsmann, W., and Dubelaar, M. (1992) Carnitine requirement of vascular endothelial and smooth muscle cells in imminent ischemia. *Molecular and cellular biochemistry* **116**, 125-129
75. Fukami, K., Yamagishi, S., Sakai, K., Kaida, Y., Yokoro, M., Ueda, S., Wada, Y., Takeuchi, M., Shimizu, M., Yamazaki, H., and Okuda, S. (2015) Oral L-carnitine supplementation increases trimethylamine-N-oxide but reduces markers of vascular injury in hemodialysis patients. *Journal of cardiovascular pharmacology* **65**, 289-295
76. Koeth, R. A., Lam-Galvez, B. R., Kirsop, J., Wang, Z., Levison, B. S., Gu, X., Copeland, M. F., Bartlett, D., Cody, D. B., Dai, H. J., Culley, M. K., Li, X. S., Fu, X., Wu, Y., Li, L., DiDonato, J. A., Tang, W. H. W., Garcia-Garcia, J. C., and Hazen, S. L. (2019) l-Carnitine in omnivorous diets induces an atherogenic gut microbial pathway in humans. *J Clin Invest* **129**, 373-387
77. Nagase, T., Miyajima, N., Tanaka, A., Sazuka, T., Seki, N., Sato, S., Tabata, S., Ishikawa, K., Kawarabayasi, Y., Kotani, H., and et al. (1995) Prediction of the coding sequences of unidentified human genes. III. The coding sequences of 40 new genes (KIAA0081-KIAA0120) deduced by analysis of cDNA clones from human cell line KG-1. *DNA research : an international journal for rapid publication of reports on genes and genomes* **2**, 37-43
78. Song, J., Yang, W., Shih Ie, M., Zhang, Z., and Bai, J. (2006) Identification of BCOX1, a novel gene overexpressed in breast cancer. *Biochimica et biophysica acta* **1760**, 62-69
79. Guo, J., Wang, M., and Liu, X. (2015) MicroRNA-195 suppresses tumor cell proliferation and metastasis by directly targeting BCOX1 in prostate carcinoma. *Journal of experimental & clinical cancer research : CR* **34**, 91
80. Liu, T., Zhang, X. Y., He, X. H., Geng, J. S., Liu, Y., Kong, D. J., Shi, Q. Y., Liu, F., Wei, W., and Pang, D. (2014) High levels of BCOX1 expression are associated with poor prognosis in patients with invasive ductal carcinomas of the breast. *PloS one* **9**, e86952
81. Cui, H., Lan, X., Lu, S., Zhang, F., and Zhang, W. (2017) Bioinformatic prediction and functional characterization of human KIAA0100 gene. *Journal of Pharmaceutical Analysis* **7**, 10-18
82. Zhong, Z., Pannu, V., Rosenow, M., Stark, A., and Spetzler, D. (2018) KIAA0100 Modulates Cancer Cell Aggression Behavior of MDA-MB-231 through Microtubule and Heat Shock Proteins. *Cancers* **10**, 180
83. Arnesen, T., Gromyko, D., Pendino, F., Ryningen, A., Varhaug, J. E., and Lillehaug, J. R. (2006) Induction of apoptosis in human cells by RNAi-mediated knockdown of hARD1 and NATH, components of the protein N-alpha-acetyltransferase complex. *Oncogene* **25**, 4350-4360
84. Arnesen, T., Anderson, D., Baldersheim, C., Lanotte, M., Varhaug, J. E., and Lillehaug, J. R. (2005) Identification and characterization of the human ARD1-NATH protein acetyltransferase complex. *The Biochemical journal* **386**, 433-443
85. Foyn, H., Van Damme, P., Stove, S. I., Glomnes, N., Evjenth, R., Gevaert, K., and Arnesen, T. (2013) Protein N-terminal acetyltransferases act as N-terminal propionyltransferases in vitro and in vivo. *Molecular & cellular proteomics : MCP* **12**, 42-54
86. Di Gennaro, E., Bruzzese, F., Caraglia, M., Abruzzese, A., and Budillon, A. (2004) Acetylation of proteins as novel target for antitumor therapy. *Amino acids* **26**, 435-441

87. Fluge, O., Bruland, O., Akslen, L. A., Varhaug, J. E., and Lillehaug, J. R. (2002) NATH, a novel gene overexpressed in papillary thyroid carcinomas. *Oncogene* **21**, 5056-5068
88. Sugiura, N., Adams, S. M., and Corriveau, R. A. (2003) An evolutionarily conserved N-terminal acetyltransferase complex associated with neuronal development. *Journal of Biological Chemistry* **278**, 40113-40120
89. Sugiura, N., Patel, R. G., and Corriveau, R. A. (2001) N-methyl-D-aspartate receptors regulate a group of transiently expressed genes in the developing brain. *Journal of Biological Chemistry* **276**, 14257-14263
90. Cheng, H., Dharmadhikari, A. V., Varland, S., Ma, N., Domingo, D., Kleyner, R., Rope, A. F., Yoon, M., Stray-Pedersen, A., Posey, J. E., Crews, S. R., Eldomery, M. K., Akdemir, Z. C., Lewis, A. M., Sutton, V. R., Rosenfeld, J. A., Conboy, E., Agre, K., Xia, F., Walkiewicz, M., Longoni, M., High, F. A., van Slegtenhorst, M. A., Mancini, G. M. S., Finnila, C. R., van Haeringen, A., den Hollander, N., Ruivenkamp, C., Naidu, S., Mahida, S., Palmer, E. E., Murray, L., Lim, D., Jayakar, P., Parker, M. J., Giusto, S., Stracuzzi, E., Romano, C., Beighley, J. S., Bernier, R. A., Küry, S., Nizon, M., Corbett, M. A., Shaw, M., Gardner, A., Barnett, C., Armstrong, R., Kassahn, K. S., Van Dijck, A., Vandeweyer, G., Kleefstra, T., Schieving, J., Jongmans, M. J., de Vries, B. B. A., Pfundt, R., Kerr, B., Rojas, S. K., Boycott, K. M., Person, R., Willaert, R., Eichler, E. E., Kooy, R. F., Yang, Y., Wu, J. C., Lupski, J. R., Arnesen, T., Cooper, G. M., Chung, W. K., Gecz, J., Stessman, H. A. F., Meng, L., and Lyon, G. J. (2018) Truncating Variants in NAA15 Are Associated with Variable Levels of Intellectual Disability, Autism Spectrum Disorder, and Congenital Anomalies. *The American Journal of Human Genetics* **102**, 985-994
91. Bilban, M., Hofbauer, R., Hofer-Litzlbauer, E., Kienesberger, K., Molzer, S., and Wagner, O. (2017) Microarray analysis of carnitine deficiency and supplementation reveals that the human
genome is heavily influenced on the transcriptional level.
92. Gruenbaum, Y., Margalit, A., Goldman, R. D., Shumaker, D. K., and Wilson, K. L. (2005) The nuclear lamina comes of age. *Nature reviews. Molecular cell biology* **6**, 21-31
93. Gruenbaum, Y., and Foisner, R. (2015) Lamins: nuclear intermediate filament proteins with fundamental functions in nuclear mechanics and genome regulation. *Annual review of biochemistry* **84**, 131-164
94. Liu, N. A., Sun, J., Kono, K., Horikoshi, Y., Ikura, T., Tong, X., Haraguchi, T., and Tashiro, S. (2015) Regulation of homologous recombinational repair by lamin B1 in radiation-induced DNA damage. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology* **29**, 2514-2525
95. Lukasova, E., Kovar ik, A., Bac ikova, A., Falk, M., and Kozubek, S. (2017) Loss of lamin B receptor is necessary to induce cellular senescence. *The Biochemical journal* **474**, 281-300
96. Tsai, M.-Y., Wang, S., Heidinger, J. M., Shumaker, D. K., Adam, S. A., Goldman, R. D., and Zheng, Y. (2006) A mitotic lamin B matrix induced by RanGTP required for spindle assembly. *Science* **311**, 1887-1893
97. Yeh, C. H., Kuo, P. L., Wang, Y. Y., Wu, Y. Y., Chen, M. F., Lin, D. Y., Lai, T. H., Chiang, H. S., and Lin, Y. H. (2015) SEPT12/SPAG4/LAMINB1 complexes are required for maintaining the integrity of the nuclear envelope in postmeiotic male germ cells. *PloS one* **10**, e0120722

98. Padiath, Q. S., Saigoh, K., Schiffmann, R., Asahara, H., Yamada, T., Koeppen, A., Hogan, K., Ptacek, L. J., and Fu, Y. H. (2006) Lamin B1 duplications cause autosomal dominant leukodystrophy. *Nature genetics* **38**, 1114-1123
99. Brussino, A., Vaula, G., Cagnoli, C., Mauro, A., Pradotto, L., Daniele, D., Di Gregorio, E., Barberis, M., Arduino, C., Squadrone, S., Abete, M. C., Migone, N., Calabrese, O., and Brusco, A. (2009) A novel family with Lamin B1 duplication associated with adult-onset leucoencephalopathy. *Journal of neurology, neurosurgery, and psychiatry* **80**, 237-240
100. Sun, S., Xu, M. Z., Poon, R. T., Day, P. J., and Luk, J. M. (2010) Circulating Lamin B1 (LMNB1) biomarker detects early stages of liver cancer in patients. *Journal of proteome research* **9**, 70-78
101. Radu, M., and Chernoff, J. (2009) The DeMSTification of mammalian Ste20 kinases. *Current biology : CB* **19**, R421-425
102. Taylor, L. K., Wang, H. C., and Erikson, R. L. (1996) Newly identified stress-responsive protein kinases, Krs-1 and Krs-2. *Proceedings of the National Academy of Sciences of the United States of America* **93**, 10099-10104
103. Ura, S., Masuyama, N., Graves, J. D., and Gotoh, Y. (2001) Caspase cleavage of MST1 promotes nuclear translocation and chromatin condensation. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 10148-10153
104. Creasy, C. L., and Chernoff, J. (1995) Cloning and characterization of a human protein kinase with homology to Ste20. *The Journal of biological chemistry* **270**, 21695-21700
105. Meng, F., Zhou, R., Wu, S., Zhang, Q., Jin, Q., Zhou, Y., Plouffe, S. W., Liu, S., Song, H., Xia, Z., Zhao, B., Ye, S., Feng, X. H., Guan, K. L., Zou, J., and Xu, P. (2016) Mst1 shuts off cytosolic antiviral defense through IRF3 phosphorylation. *Genes & development* **30**, 1086-1100
106. Zhou, D., Medoff, B. D., Chen, L., Li, L., Zhang, X.-f., Praskova, M., Liu, M., Landry, A., Blumberg, R. S., and Boussiotis, V. A. (2008) The Nore1B/Mst1 complex restrains antigen receptor-induced proliferation of naive T cells. *Proceedings of the National Academy of Sciences*, pnas. 0810773105
107. Dong, Y., Du, X., Ye, J., Han, M., Xu, T., Zhuang, Y., and Tao, W. (2009) A cell-intrinsic role for Mst1 in regulating thymocyte egress. *The Journal of Immunology*, jimmunol. 0900678
108. Abdollahpour, H., Appaswamy, G., Kotlarz, D., Diestelhorst, J., Beier, R., Schaffer, A. A., Gertz, E. M., Schambach, A., Kreipe, H. H., Pfeifer, D., Engelhardt, K. R., Rezaei, N., Grimbacher, B., Lohrmann, S., Sherkat, R., and Klein, C. (2012) The phenotype of human STK4 deficiency. *Blood* **119**, 3450-3457
109. Nehme, N. T., Schmid, J. P., Debeurme, F., Andre-Schmutz, I., Lim, A., Nitschke, P., Rieux-Laucat, F., Lutz, P., Picard, C., Mahlaoui, N., Fischer, A., and de Saint Basile, G. (2012) MST1 mutations in autosomal recessive primary immunodeficiency characterized by defective naive T-cell survival. *Blood* **119**, 3458-3468
110. Waterman, M., Xu, W., Stempak, J. M., Milgrom, R., Bernstein, C. N., Griffiths, A. M., Greenberg, G. R., Steinhart, A. H., and Silverberg, M. S. (2011) Distinct and overlapping genetic loci in Crohn's disease and ulcerative colitis: correlations with pathogenesis. *Inflammatory bowel diseases* **17**, 1936-1942

111. Nimmo, E. R., Prendergast, J. G., Aldhous, M. C., Kennedy, N. A., Henderson, P., Drummond, H. E., Ramsahoye, B. H., Wilson, D. C., Semple, C. A., and Satsangi, J. (2012) Genome-wide methylation profiling in Crohn's disease identifies altered epigenetic regulation of key host defense mechanisms including the Th17 pathway. *Inflammatory bowel diseases* **18**, 889-899
112. Ready, D., Yagiz, K., Amin, P., Yildiz, Y., Funari, V., Bozdag, S., and Cinar, B. (2017) Mapping the STK4/Hippo signaling network in prostate cancer cell. *PloS one* **12**, e0184590
113. Li, W., Xiao, J., Zhou, X., Xu, M., Hu, C., Xu, X., Lu, Y., Liu, C., Xue, S., Nie, L., Zhang, H., Li, Z., Zhang, Y., Ji, F., Hui, L., Tao, W., Wei, B., and Wang, H. (2015) STK4 regulates TLR pathways and protects against chronic inflammation-related hepatocellular carcinoma. *The Journal of clinical investigation* **125**, 4239-4254
114. Gutierrez-Ruiz, M., Bucio, L., Souza, V., Gomez, J., Campos, C., and Carabez, A. (1994) Expression of some hepatocyte-like functional properties of WRL-68 cells in culture. *In Vitro Cellular & Developmental Biology-Animal* **30**, 366-371
115. Yuan, J. S., Reed, A., Chen, F., and Stewart, C. N. (2006) Statistical analysis of real-time PCR data. *BMC bioinformatics* **7**, 85
116. Pfaffl, M. W. (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic acids research* **29**, e45
117. Frank, S. G., and Bernard, P. S. (2004) Profiling breast cancer using real-time quantitative PCR. in *Rapid Cycle Real-Time PCR—Methods and Applications*, Springer. pp 95-106
118. Pfaffl, M. W. (2004) Real-time RT-PCR: neue Ansätze zur exakten mRNA Quantifizierung. *BIOspektrum* **1**, 92-95
119. Pfaffl, M. W., Horgan, G. W., and Dempfle, L. (2002) Relative expression software tool (REST©) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. *Nucleic acids research* **30**, e36-e36
120. Michaud, D. S., Augustsson, K., Rimm, E. B., Stampfer, M. J., Willet, W. C., and Giovannucci, E. (2001) A prospective study on intake of animal products and risk of prostate cancer. *Cancer causes & control : CCC* **12**, 557-567
121. Giovannucci, E., Rimm, E. B., Colditz, G. A., Stampfer, M. J., Ascherio, A., Chute, C. G., and Willett, W. C. (1993) A prospective study of dietary fat and risk of prostate cancer. *Journal of the National Cancer Institute* **85**, 1571-1579
122. Roberts, C. T., Jr. (2004) IGF-1 and prostate cancer. *Novartis Foundation symposium* **262**, 193-199; discussion 199-204, 265-198

7. Appendix

7.1. Abbreviations

ADLD	autosomal dominant leukodystrophy with autonomic disease
BCL2	B-cell lymphoma 2
bp	basepairs
BCOX1	breast cancer overexpressed gene 1, alternative spliced form of KIAA0100
cDNA	complementary deoxyribonucleic acid
CoA	coenzyme A
Dia	dialyzed
FAS	fetal alcohol syndrome
FCS	fetal calf serum
FOXO1	forkhead box protein O1
hARD1	human arrest-defective-1
HCV	hepatitis C virus
hrs	hours
IL7R	Interleukin-7 Receptor
kb	kilobase
KIAA0100	BCOX1
LMNB1	lamin protein B1
ml	mililitre
mRNA	micro ribonucleic acid
micro-RNA-195	micro ribonucleic acid – 195
MST1	STK4
NATH	N (alpha-)acetyltransferase 15, TBDN100
n.a.	not available
OCTN2	organic cation transporter 2
PCR	polymerase chain reaction
qPCR	real-time quantitative polymerase chain reaction
TBDN100	transcriptional coactivator tubedown-100, NATH
SKMC	human primary skeletal muscle cell
STK4	serine/threonine kinase4, MST1
SYBR Green	cyanine dye
WRL68	human epithelial heterploid liver cell line
µl	microlitre
µM	micromolar
3’ UTR	3’ untranslated region

7.2. Zusammenfassung

Zusammengefasst zeigt diese Arbeit erstmalig organspezifische Unterschiede in der Genexpression nach L-Carnitin Entzug und anschließender Beimengung von L-Carnitin, auf. Daneben liefert sie weitere experimentelle Beweise für die Effekte von L-Carnitin auf die Genexpression. In Bezug auf die statistischen Daten, zeigen alle untersuchten Gene einen signifikanten Einfluss von L-Carnitin auf die Amplifikationsrate. Das nennenswerteste Ergebnis ist der Unterschied zwischen der Genexpression von TBDN100 in den WRL68 Leberzellen und den SKMC. Daneben sei noch die Korrelation zwischen der Herunterregulierung der Genexpression von TBDN100 und der Beimengung des L-Carnitin-Zusatzes zu erwähnen. Einen weiteren wichtigen Aspekt stellt die signifikant positive Korrelation von BCOX1 und dem 40µM L-Carnitin-Zusatz als Kultivierungsbedingung dar. Vor allem die Genexpressionsmenge von BCOX1 in der 40µM Probe der SKMC zeigt einen erhöhten Wert. Alles in allem, werden weitere Untersuchungen notwendig sein, um die organspezifischen transkriptionellen Effekte des nutrigenomischen Faktors L-Carnitin zu zeigen.