UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

Tamara Teodoro Araújo

**Proteomic analysis of mitochondria from rat liver exposed to chronic
fluoride exposure in two experimental periods**

**Análise proteômica das mitocôndrias de fígados de ratos expostos à
exposição crônica por fluoreto em dois períodos experimentais**

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Dissertation presented to the Bauru School of Dentistry of the University of São Paulo to obtain the degree of Master in Science in the Applied Dental Science Program, Stomatology and Oral Biology concentration area.

Supervisor: Prof. Dr^a Marília Afonso Rabelo Buzalaf

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“Só o riso, o amor e o prazer merecem revanche.

O resto, mais que perda de tempo...

É perda de vida “.

(Chico Xavier)

RESUMO

O íon fluoreto (F), quando ingerido em excesso, pode alterar várias funções celulares. Como o fígado é um órgão de alta atividade metabólica, tornou-se alvo de investigações sobre os efeitos colaterais do F, relacionados a alterações na expressão de proteínas metabólicas, aumento do estresse oxidativo e alterações na função mitocondrial. Assim, o objetivo do presente trabalho foi avaliar o perfil de expressão proteica de mitocôndrias hepáticas de ratos expostos cronicamente a duas concentrações de F na água de beber (15 ou 50 mgF / L) por 20 ou 60 dias. Trinta e seis amostras de fígado de ratos *Wistar* machos com 21 dias de idade foram divididos em 6 grupos (n= 6 animais por grupo) de acordo com a concentração de F administrada em água potável (0, 15 ou 50 mg / L) e o tratamento tempo (20 ou 60 dias). As mitocôndrias foram extraídas do tecido hepático e preparadas para análise proteômica quantitativa sem marcadores (Xevo Q-TOF, Waters). O software PLGS foi utilizado para detectar mudanças na expressão de proteínas entre os diferentes grupos. A maioria das alterações foi observada nas vias metabólicas como glicólise, gliconeogênese, β -oxidação, ciclo da ureia, ciclo dos ácidos tricarboxílicos e cadeia de transporte de elétrons, além de alterações nas proteínas envolvidas no sistema antioxidante e homeostase do cálcio. A dose de 15 mgF/L, quando administrada por 20 dias, reduziu a glicólise, que foi contrabalançada pelo aumento de outras vias energéticas. Aos 60 dias, no entanto, um aumento em todas as vias de energia foi observado. Por outro lado, a dose de 50 mgF/L, quando administrada por 20 dias, reduziu as enzimas envolvidas em todas as vias energéticas, indicando menor taxa de produção de energia, com menor geração de ROS e consequente redução de enzimas antioxidantes. No entanto, quando a dose de 50 mgF/L foi administrada por 60 dias, observou-se um aumento no metabolismo energético, mas, em geral, não foram observadas alterações nas enzimas antioxidantes. Com exceção do grupo tratado com 50 mgF/L por 20 dias, todos os demais grupos apresentaram alterações nas proteínas na tentativa de manter a homeostase do cálcio e evitar a apoptose. Assim, os resultados sugerem que o organismo parece se adaptar aos efeitos do F ao longo do tempo, ativando vias para reduzir a toxicidade desse íon. Em última análise, nossos achados corroboram com a segurança do uso do flúor no controle da cárie.

Palavras-chave: Fluoreto. Fígado. Mitocôndria. Metabolismo energético. Sistema antioxidante.

ABSTRACT

Proteomic analysis of mitochondria from rat liver exposed to chronic fluoride exposure in two experimental periods.

The fluoride ion (F), when ingested in excess, may alter various cellular functions. As liver is an organ of high metabolic activity, it has become the target of investigations on the side effects of F, related to changes in the expression of metabolic proteins, increase in oxidative stress and changes in mitochondrial function. Thus, the objective of the present work was to evaluate the protein expression profile of liver mitochondria from rats chronically exposed to two F concentrations through the drinking water (15 or 50 mgF/L) for 20 or 60 days. Thirty-six liver samples from 21-day-old male *Wistar* rats were divided into 6 groups (n= 6 animals per group) according to the concentration of F administered in drinking water (0, 15 or 50 mg/L) and the treatment time (20 or 60 days). Mitochondria were extracted from hepatic tissue and prepared for quantitative label-free proteomic analysis (Xevo Q-TOF, Waters). PLGS software was used to detect changes in protein expression among the different groups. Most of the changes were observed in the metabolic pathways such as glycolysis, gluconeogenesis, β -oxidation, urea cycle, tricarboxylic acids cycle and electron transport chain, in addition alterations in proteins involved in the antioxidant system, calcium homeostasis and apoptosis. The dose of 15 mgF/L, when administered for 20 days, reduced glycolysis, which was counterbalanced by an increase in other energetic pathways. At 60 days, however, an increase in all energy pathways was observed. On the other hand, the dose of 50 mgF/L, when administered for 20 days, reduced the enzymes involved in all energetic pathways, indicating a lower rate of energy production, with less generation of ROS and consequent reduction of antioxidant enzymes. However, when the 50 mgF/L dose was administered for 60 days, an increase in energy metabolism was seen but in general no changes were observed in the antioxidant enzymes. Except for the group treated with 50 mgF/L for 20 days, all the other groups had alterations in proteins in attempt to maintain calcium homeostasis and avoid apoptosis. Thus, the results suggest that the organism seems to adapt to the effects of F over time, activating pathways to reduce the toxicity of this ion. Ultimately, our findings corroborate the safety of the use of fluoride for caries control.

Key-words: Fluoride. Liver. Mitochondria. Energy metabolism. Antioxidant system.

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1- *I*ntroduction

1 INTRODUCTION

The fluoride ion (F) is derived from the chemical element fluorine, a gaseous element from the group of halogens (VII), extremely reactive and electronegative. These characteristics make easier its complexation with other elements of the periodic table, thus forming organic and/or inorganic compounds (Smith F, 1996; Barbier *et al.*, 2010). When naturally identified in the soil, water and vapors of volcanic eruptions, F is always associated with another element and is never in its free form (Buzalaf, 2013). Due to this, in this work we will mention the ion F but not the chemical element fluorine.

As F is naturally present in the environment, in some regions of the world it can be found in high concentrations in the groundwater, which may result in excessive concentrations in the water supply. In these situations, it is estimated that the intake of F can reach up to 27 mg/day (Dey *et al.*, 2011). Because of these elevated concentrations in natural environments, the World Health Organization (WHO) has established a limiting concentration of 1.5 mg F/L in the drinking water as a safe limit, and the average daily intake of F should not exceed 2 mg/day. High concentrations of F can also be seen in some regions of Brazil (Pessan *et al.*, 2008; De Souza *et al.*, 2013), as well as in other areas of the world such as India (Hussain *et al.*, 2010). Due to the established effectiveness for the control of dental caries, supplementation of F in the drinking water of several municipalities has become frequent. Since F is available in fluoridated water, food and supplements, its daily intake can easily exceed the WHO recommended limit, leading to toxic responses in the body (Buzalaf, 2018).

When F is ingested, approximately 25% is absorbed by the gastric mucosa in the form of HF, a process inversely related to the pH. The fraction that is not absorbed by the stomach will be absorbed in the intestine. In this case F passes in the space between the cells of the intestinal mucosa, which does not depend on the pH. Due to the rapid absorption, plasma F concentration rises rapidly, reaching a peak between 30-45 minutes after ingestion. Much of the circulating F will be incorporated into the mineralized tissues and only a small fraction is taken up by the soft tissues. In the latter, the entry or exit of F from the cells depends on the pH gradient between the intra- and extracellular compartments, since the cells are permeable to HF. Absorbed F that is not incorporated into the body will be excreted mainly through the urinary tract, a phenomenon that is also dependent on the pH of the urine (Buzalaf e Whitford, 2011; Buzalaf, 2013). Thus, after absorption, F is rapidly distributed to the body and can reach all

tissues and cell types. Thus, depending on the amount of F ingested, duration of ingestion, solubility of the compounds containing it and individual factors, F can disturb the homeostasis of the organism (Buzalaf e Whitford, 2011).

For many decades, several studies have been conducted in attempt to investigate the effects of F in the organism. Many in vitro laboratory studies, as well as in vivo investigations, conducted both with animals and humans have shown adverse effects caused by excessive ingestion of F, which are related to the amount and time of exposure (for review see (Barbier *et al.*, 2010; Wei *et al.*, 2018; Zuo *et al.*, 2018). The most well documented adverse effect of F is fluorosis (Buzalaf, 2013). It can occur in three forms according to the affected tissues, namely: dental, skeletal and non-skeletal that affects soft tissues such as liver, kidney, lung, muscle, blood, cells, gastrointestinal mucosa. In these tissues, F affects several proteins and enzymes, and interferes in distinct molecular processes. Among these are cell cycle, ion transport, apoptosis, signaling pathways and metabolism of free radicals. These effects have been described in several organs and tissues such as liver, kidney, heart, brain, muscle and intestine (Whitford, 1996; Chlubek *et al.*, 2003; Shanthakumari *et al.*, 2004; Strunecka, 2007; Strunecka *et al.*, 2007; Xiong *et al.*, 2007; Blaszczyk *et al.*, 2008; Chouhan e Flora, 2008; Kobayashi *et al.*, 2009; Barbier *et al.*, 2010; Inkielewicz-Stepniak e Czarnowski, 2010; Dey *et al.*, 2011; Pereira *et al.*, 2013; Lima Leite *et al.*, 2014; Pereira *et al.*, 2016; Melo *et al.*, 2017; Dionizio, A. *et al.*, 2018; Dionizio, A. S. *et al.*, 2018; Malvezzi *et al.*, 2018; Pereira *et al.*, 2018).

Experimental studies to evaluate F toxicity are usually performed in animals, especially in rodents where drinking water supplemented with various concentrations of F is commonly used for distinct periods, thus allowing assessment of the effects of short- and long-term exposure to this ion. When rodents are used as a model, it is important to bear in mind that they metabolize F 5 times faster than humans (Dunipace *et al.*, 1995). This means that, e.g., the F concentration of 15 mgF/L in the drinking water for rats corresponds to approximately water containing 3 mgF/L for humans. This concentration can be naturally found in the drinking water of endemic regions of fluorosis and is sufficient to cause hepatic changes, since the liver stands out as the main organ of the body involved in the detoxification of the organism (Dabrowska *et al.*, 2006; Xiong *et al.*, 2007; Pereira *et al.*, 2013; Iano *et al.*, 2014; Yan e Xie, 2015; Zhou *et al.*, 2015; Dionizio, A. *et al.*, 2018; Khan *et al.*, 2018; Pereira *et al.*, 2018). The liver has a high metabolic rate, which shares substrates and energy in addition to receiving the nutrients absorbed in the digestive system, processing them, storing them and synthesizing substances that are transported to other parts of the body. It is an organ that neutralizes and eliminates toxic substances (Merrick, 2006). Some alterations may be due to the metabolism of liver tissue itself

in order to eliminate toxic compounds, which can lead to cellular dysfunction and oxidative stress, and, depending on genetic factors, may give rise to a greater or lesser degree of cellular stress (Stirnimann *et al.*, 2010). Studies involving enzymes related to hepatic metabolism such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have shown alterations in the presence of high concentrations of F and may indicate their direct action in the organ with impaired hepatic functions (Michael *et al.*, 1996; Pereira *et al.*, 2018).

The toxic effects of F have often been related as an important factor causing oxidative stress, defined as the sum of factors that cause an imbalance between the antioxidant enzymes and the reactive oxygen species (ROS) (Dabrowska *et al.*, 2004; Suzuki *et al.*, 2015; Zhou *et al.*, 2015; Pereira *et al.*, 2016). Mitochondria are considered the main sources of ROS and this has been related to the increase in the production of superoxide anions. This excessive production can contribute to mitochondrial damage and be an important mechanism in redox signaling of the organelle (Brownlee, 2001; Droge, 2002; Balaban *et al.*, 2005; Murphy, 2009; Sun *et al.*, 2014; Dionizio, A. *et al.*, 2018).

Mitochondria are organelles with diverse forms and functions, among which the main one is bioenergetics that involves the transformation of energy derived from oxidation/reduction reactions into chemical energy (ATP). ATP is produced mainly in the electron transport chain and perturbations in this process can lead to excessive production of ROS, which in turn can alter calcium signaling and induce apoptosis (Fagian *et al.*, 1990; Castilho *et al.*, 1995; Castilho *et al.*, 1996; Kowaltowski *et al.*, 1996; Komarova *et al.*, 2000; Yan e Xie, 2015). There are several reports indicating disturbs in energy metabolism induced by F, leading to the generation of oxidative stress and eventually apoptosis, depending on the dose (Barbier *et al.*, 2010; Fina *et al.*, 2014; Jothiramajayam *et al.*, 2014; Pereira *et al.*, 2018; Wei *et al.*, 2018). In addition, F has been reported to act directly as an uncoupling agent on mitochondria (Ligeret *et al.*, 2004), which promotes the opening of the transition pore of mitochondrial permeability (TPMP) and increases the disruption of the mitochondrial membrane potential (MMP) and then provokes the release of Cyt c, leading to caspase-3 cleavage and finally apoptosis (Wei *et al.*, 2018). Recent studies from our research group employing proteomic tools reported that different doses of F alter the expression of several mitochondrial proteins. Both in liver, muscle and intestine of rats and mice, exposure to F provoked alterations in energy metabolism, mitochondrial dysfunction and oxidative stress (Kobayashi *et al.*, 2009; Lima Leite *et al.*, 2014; Lobo *et al.*, 2015; Pereira *et al.*, 2016; Dionizio, A. S. *et al.*, 2018; Malvezzi *et al.*, 2018; Pereira *et al.*, 2018). Similar findings were reported by Lima Leite *et al.* (2014). However, in these studies, proteomic analysis was performed in the

homogenate of the tissues. In the present study, we isolated the mitochondrial fraction of the liver of rats, since our focus was to detect changes in mitochondrial proteins induced by F.

This dissertation is presented in the form of an article, in which we evaluated the effect of the different concentrations of F administered for 20 or 60 days in the proteomic profile of mitochondria of liver of rats.

2- Article

2 ARTICLE

Article formatted according to Ecotoxicology and Environmental Safety

Changes in energy metabolism induced by fluoride: insights from inside the mitochondria

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ABSTRACT

The mechanisms involved in changes in energy metabolism caused by excessive exposure to fluoride (F) are not completely understood. **Aim:** The present study employed proteomic tools to investigate the molecular mechanisms underlying the dose- and time-dependency of the effects of F in the rat liver mitochondria. **Materials and methods:** Thirty-six male *Wistar* rats received water containing 0, 15 or 50 mgF/L for 20 or 60 days. Rat liver mitochondria were isolated and the proteome profiles were examined using label-free quantitative nLC–MS/MS. PLGS software was used to detect changes in protein expression among the different groups. The bioinformatics analysis was done using the software CYTOSCAPE® 3.0.7 (Java®) with the aid of ClueGo plugin. **Results:** The dose of 15 mgF/L, when administered for 20 days, reduced glycolysis, which was counterbalanced by an increase in other energetic pathways. At 60 days, however, an increase in all energy pathways was observed. On the other hand, the dose of 50 mgF/L, when administered for 20 days, reduced the enzymes involved in all energetic pathways, indicating a lower rate of energy production, with less generation of ROS and consequent reduction of antioxidant enzymes. However, when the 50 mgF/L dose was administered for 60 days, an increase in energy metabolism was seen but in general no changes were observed in the antioxidant enzymes. Except for the group treated with 50 mgF/L for 20 days, all the other groups had alterations in proteins in attempt to maintain calcium homeostasis and avoid apoptosis. **Conclusion:** The results suggest that the organism seems to adapt to the effects of F over time, activating pathways to reduce the toxicity of this ion. Ultimately, our findings corroborate the safety of the use of F for caries control.

Keywords: Fluoride. Mitochondria. Liver. Energy metabolism. Oxidative stress.

INTRODUCTION

Fluoride (F) is widely available in the nature and has been employed with great success to control dental caries all over the world. Dental fluorosis is the only recognized side-effect of the appropriate use of F in dentistry, when this ion is included in the drinking water and in dentifrices (Buzalaf, 2018). However, excessive F intake for long periods may result in skeletal fluorosis and damage to soft tissues, due to disturbances in various cell processes and pathways, such signaling pathways, ionic transport, energy metabolism and redox status (Barbier et al., 2010). The cellular effects of F have been extensively investigated in the last two decades with the advent of proteomic analysis. Alterations have been reported in different organs and tissues, such as liver (Khan et al., 2018; Lobo et al., 2015; Pereira et al., 2018; Pereira et al., 2013), kidney (Carvalho et al., 2013; Kobayashi et al., 2009), muscle (Lima Leite et al., 2014; Malvezzi et al., 2019) and intestine (Dionizio et al., 2018; Melo et al., 2017). Among these, liver has probably been the most investigated one, since it is the central organ involved in the metabolism. Alterations in mitochondrial proteins involved in energy metabolism are the most common finding of the studies that employ proteomic tools to evaluate the effects of exposure to F in the liver (Khan et al., 2018; Lobo et al., 2015; Pereira et al., 2018; Pereira et al., 2013). Remarkably, these effects are reported to be dose- and time-dependent (Dabrowska et al., 2006; Khan et al., 2018; Pereira et al., 2018), which makes comparison of studies with different designs a difficult task.

Since F alters the energy metabolism, a common effect associated with excessive exposure to F is oxidative stress (Barbier et al., 2010; Zuo et al., 2018) that, if not counteracted, can lead to apoptosis (Wei et al., 2018). Mitochondria is a key organelle involved in all these events. Thus, the aim of the present study was to focus on the alterations in proteins involved in the energy metabolism, upon exposure to F. For this, we performed proteomic analysis of

mitochondria isolated from the rat liver. Moreover, different doses and periods of exposure to F were also investigated.

MATERIAL AND METHODS

Animals and treatment

The experimental protocols were approved by the Ethics Committee on animal experimentation of Bauru School of Dentistry, University of São Paulo (protocol: 009/2017). Male *Wistar* rats (3 weeks of age – *Rattus Norvegicus*) were randomized into two groups (n= 18/group) according to the treatment periods (20 or 60 days). Each group was further subdivided into 3 subgroups (n= 6/subgroup) according to F concentrations (as sodium fluoride) administered in the drinking water: 0 mg/L (control), 15 mg/L or 50 mg/L. These concentrations were used because they can be naturally found in areas of endemic fluorosis and lead to plasma F levels in the rats that are correspondent to those found in humans drinking water containing 3 and 10 mgF/L, respectively (Dunipace et al., 1995). The animals were housed in pairs in standard cages and received diet and water *ad libitum*. The room was maintained with light/dark cycle of 12 hours and temperature of 23±1°C. At the end of the experimental period, the animals received an intramuscular injection of anesthetic (ketamine hydrochloride) and muscle relaxant (xylazine hydrochloride). The liver was collected and washed with ice cold buffer (100 mM Tris, 1 mM EDTA, 1 mM PMSF, pH 7.4), followed immediately by mitochondrial isolation.

Isolation of Liver Mitochondria

The isolation was performed as previously described (Frezza et al., 2007). For isolation, the MSTE buffer (0.23 M mannitol, 0.07 M sucrose, 10 mM Tris-HCl, 1 mM EDTA, pH 7) was used. Eighteen mL of MSTE buffer at 4°C were added to 2 g of liver and homogenized.

Homogenate was centrifuged at 700 g for 10 minutes at 4°C to break the cell membranes. The supernatant was collected and centrifuged at 8000 g for 10 minutes at 4°C. At the end, the formation of a heart-shaped pellet was observed. The supernatant was discarded, the pellet was resuspended in 1 ml of cold MSTE buffer and stored at -80°C.

Preparation of mitochondria for Proteomic Analysis

Protein extraction was performed as previously described (Dionizio et al., 2018). Briefly, the proteins were extracted using the lysis buffer (7 M urea, 2 M thiourea, 40 mM DTT, all diluted in AMBIC solution) under constant stirring at 4°C. Samples were centrifuged at 21952 g for 30 minutes at 4°C and the supernatant was collected. Protein quantification was performed (Bradford, 1976). Samples from 2 animals were pooled in order that analyses were carried out in biological triplicates. To 50 µL of each sample (containing 50 µg protein), 25 µL of 0.2% Rapigest (WATERS cat#186001861) were added, followed by agitation, and then 10 µL of 50 mM ammonium bicarbonate were added. Samples were incubated for 30 minutes at 37 °C. They were then reduced (2.5 µL 100 mM dithiothreitol, BIORAD, cat# 161–0611) and alkylated (2.5 µL 300 mM iodoacetamide, GE, cat# RPN 6302 V) under dark at room temperature for 30 minutes. Digestion was performed at 37°C overnight by adding 100 ng trypsin (PROMEGA, cat #V5280). After digestion, 10 µL of 5% trifluoroacetic acid were added, incubated for 90 minutes at 37°C and the sample was centrifuged (21952 g at 6°C for 30 minutes). The supernatant was purified using C 18 Spin columns (PIERCE, cat #89870). Samples were resuspended in 200 µL of 3% acetonitrile.

LC-MS/MS Analysis and bioinformatics analysis

The peptides identification was performed on a nanoAcquity UPLC-Xevo QTof MS system (Waters, Manchester, UK), exactly as previously described (Lima Leite et al., 2014).

Difference in expression among the groups was obtained using the PLGS software and expressed as $p < 0.05$ for down-regulated proteins and $1 - p > 0.95$ for up-regulated proteins. The bioinformatics analysis was done using the software CYTOSCAPE® 3.0.7 (Java®) with the aid of ClueGo plugin (Bindea et al., 2013; Bindea et al., 2009) and ClusterMarker to develop molecular interaction networks with the identified proteins.

RESULTS

Supplementary tables 1 and 2 show the proteins with difference in expression as well as the unique proteins identified in the comparisons 15 mgF/L vs. control and 50 mgF/L vs. control, respectively, for the groups treated for 20 days. Supplementary tables 3 and 4 show the same data for the groups treated for 60 days.

For the comparison 15 mgF/L vs. control (20 days), 111 proteins were upregulated and 115 were downregulated, while 102 and 111 proteins were exclusively identified in the control and 15 mgF/L groups, respectively (Table S1). For this comparison, the terms with the highest percentage of associated genes (Figure 1A) were oxoacid metabolic process (42%), purine ribonucleoside triphosphate metabolic process (16%) and ATP metabolic process (15%). Regarding the comparison of the group treated with 50 mgF/L vs. control (20 days), only one protein (Peroxisomal acyl-coenzyme A oxidase 1; P07872) was upregulated and 200 proteins were downregulated in the first, while 238 and 25 proteins were exclusively identified in the control and 50 mgF/L groups, respectively (Table S2). The terms with the highest percentage of associated genes (Figure 1B) were carboxylic acid metabolic process (55%), purine ribonucleoside triphosphate metabolic process (20%) and ATP metabolic process (17%).

20 days

Figure 1. Functional distribution of proteins identified with differential expression in the rat liver mitochondria exposed to the chronic dose of (A) 15 mgF/L vs. Control Group and (B) 50 mgF/L vs. Control Group for 20 days. Categories of proteins based on GO annotation Biological Process. Terms significant ($Kappa = 0.04$) and distribution according to percentage of number of genes association. Proteins access number was provided by UNIPROT. The gene ontology was evaluated according to ClueGo® pluggins of Cytoscape® software 3.7.0.

For the groups treated for 60 days, for the comparison 15 mgF/L group vs. control, 179 proteins were upregulated and 4 were downregulated in the first. Moreover, 42 proteins were exclusively identified in the control group, while 165 proteins were exclusively identified in the group treated with 15 mgF/L (Table S3). For this comparison, the terms with the highest percentage of associated genes (Figure 2A) were carboxylic acid metabolic process (63%), electron transport chain (19%) and also coenzyme metabolic process (18%). As for the comparison of the group treated with 50 mgF/L vs. control, 57 proteins were upregulated and 33 were downregulated in the first. Besides, 64 and 46 proteins were exclusively identified in the control group and F-treated groups, respectively (Table S4). The terms with the highest percentage of associated genes (Figure 2B) were carboxylic acid metabolic process (62%) and fatty acid beta-oxidation (38%).

60 days

Figure 2. Functional distribution of proteins identified with differential expression in the rat liver mitochondria exposed to the chronic dose of (A) 15 mgF/L vs. Control Group and (B) 50 mgF/L vs. Control Group for 60 days. Categories of proteins based on GO annotation Biological Process. Terms significant ($Kappa = 0.04$) and distribution according to percentage of number of genes association. Proteins access number was provided by UNIPROT. The gene ontology was evaluated according to ClueGo® pluggins of Cytoscape® software 3.7.0.

Interaction Subnetworks

In the networks comparing 15 mgF/L vs. control groups, regardless the time of exposure, most of the proteins with altered expression interacted with 5'-AMP-activated protein kinase catalytic subunit alpha-1 (Prkaa1- P54645; Fig. 3A and 5A) and 5'-AMP-activated protein kinase subunit beta-1 (Prkab1- P21708; Fig. 3B AND 5A). Among the altered proteins involved in energy metabolism are two isoforms of L-lactate dehydrogenase (A-P04642 and B-P42123), Isocitrate dehydrogenase (P56574), Cytochrome b-c1 complex subunit 2, mitochondrial (P32551) and isoforms of ATP synthase that were increased, while Argininosuccinate synthase (P09034) and ATP synthase subunit gamma, mitochondrial (P35435) were decreased upon exposure to F for the shorter period and increased upon exposure to F for the longer period. Antioxidant proteins were also altered, such as *Prohibitin* (P67779) that was increased and *Peroxoredoxin-1* (Q63716), reduced, in the presence of F.

In the networks comparing 50 mgF/L vs. control groups, regardless the time of exposure, most of the proteins with altered expression interacted with 5'-AMP-activated protein kinase catalytic subunit alpha-1 (Prkaa1- P54645; 4A e 6A) and Mitogen-activated protein kinase 3 (P21708; Fig. 4B e 6A). The altered proteins related to energy metabolism are similar to those found in the comparison 15 mgF/L vs. control. For both periods, among the altered proteins involved in energy metabolism are L-lactate dehydrogenase B chain (P42123), three subunits of ATP synthasemmitochondrial (P15999; P10719; P19511), Argininosuccinate synthase (P09034), Pyruvate carboxylase, mitochondrial (P52873), Acetyl-CoA acetyltransferase, mitochondrial (P17764) that were decreased, while Carnitine O-palmitoyltransferase 1 liver isoform (P32198) was absent, upon exposure to F. Antioxidant proteins were also altered, such as *Prohibitin* (P67779), *Prohibitin-2* (Q5XIH7), *Peroxoredoxin-*

1 (Q63716), Glutathione peroxidase 1 (P04041) and Superoxide dismutase [Cu-Zn] (P07632) that were reduced, and Glutathione S-transferase kappa 1 (P24473) that was absent upon exposure to F (Fig. 4A, 4B and 5A). In addition, 3 isoforms of Calmodulin, interacting with Metabotropic glutamate receptor 7 (P35400) were exclusively identified upon exposure to F (Fig. 6B).

Figure 3. Subnetworks (A and B) generated by ClusterMarker® for the comparison 15 mgF/L group vs. control group for 20 days. The color of the nodes indicates the differential expression of the respective protein with its access code, available from UniProt protein database (<http://www.uniprot.org/>). The dark red and dark green nodes indicate proteins unique to the control and 15 mgF/L groups, respectively. The light red and light green nodes indicate down and upregulated proteins, respectively, in the treated groups in respect to control. The gray nodes indicate the interaction proteins that are offered by CYTOSCAPE®, which were not identified in the present study.

Figure 4. Subnetworks (A and B) generated by ClusterMarker® for the comparison 50 mgF/L vs. control group for 20 days. The color of the nodes indicates the differential expression of the respective protein with its access code, available from UniProt protein database (<http://www.uniprot.org/>). The dark red and dark green nodes indicate proteins unique to the control and 50 mgF/L groups, respectively. The light red and light green nodes indicate down and upregulated proteins, respectively, in the treated groups in respect to control. The gray nodes indicate the interaction proteins that are offered by CYTOSCAPE®, which were not identified in the present study.

Many alterations in energy metabolism were seen, on a dose- and time-dependent manner. In general, the administration of 15 mgF/L for 20 days led to a reduction in enzymes involved in glycolysis, but to an increase in enzymes involved in β -oxidation, urea cycle and electron transport chain. However, at 60 days, an increase in enzymes involved in all energetic pathways was observed (Table 1), with a concomitant increase in antioxidant enzymes and antiapoptotic proteins (Table 2).

However, when the dose of 50 mgF/L was administered for 20 days, reduction in enzymes involved in all energetic pathways was observed (Table 1) and no changes were seen in antioxidant enzymes (Table 2). At 60 days, an increase in enzymes involved in energy metabolism was observed (Table 1) but was not accompanied by changes in antioxidant enzymes (Table 2).

Table 1. Proteins related to energetic metabolism that presented significantly altered expression in the liver mitochondria of rats treated with 0 (control), 15 or 50 mgF/L for 20 or 60 days.

			20 days		60 days		20 days		60 days	
			Control	15mgF/L	Control	15mgF/L	Control	50mgF/L	Control	50mgF/L
Energy Metabolism	*Access number	Protein name description								
	P00884	Fructose-bisphosphate aldolase B		DOWN		UP		DOWN		UP
	P04797	Glyceraldehyde-3-phosphate dehydrogenase		DOWN		UP		DOWN		UP
	P16617	Phosphoglycerate kinase 1	UNIQUE							
	Q5XIV1	Phosphoglycerate kinase					UNIQUE			
	P04764	Alpha-enolase	DOWN		UNIQUE		UNIQUE			
	P12928	Pyruvate kinase PKLR	UNIQUE							
	P19629	L-lactate dehydrogenase C chain	UNIQUE		UNIQUE		UNIQUE			
	P04642	L-lactate dehydrogenase A chain	UP		UNIQUE				UNIQUE	
	P42123	L-lactate dehydrogenase B chain			UNIQUE		UNIQUE			
	P52873	Pyruvate carboxylase_ mitochondrial	UP		UP		DOWN			
	P19112	Fructose-1_6-bisphosphatase 1	DOWN		UNIQUE		DOWN		UNIQUE	
Urea Cycle	P10860	Glutamate dehydrogenase 1_ mitochondrial	UP		UP		DOWN		UP	
	P00507	Aspartate aminotransferase_ mitochondrial	UP		UNIQUE		DOWN		UP	
	P07756	Carbamoyl-phosphate synthase ammonia mitochondrial	UP				DOWN		DOWN	
	P00481	Ornithine carbamoyltransferase_ mitochondrial	UP				DOWN			
	P09034	Argininosuccinate synthase	DOWN		UNIQUE		DOWN		UNIQUE	
	P07824	Arginase-1	DOWN				DOWN			

β-oxidation	P32198	Carnitine O-palmitoyltransferase 1_ liver isoform			UNIQUE	
	P97521	Mitochondrial carnitine/acylcarnitine carrier protein	UNIQUE		UNIQUE	UNIQUE
	B3DMA2	Acyl-CoA dehydrogenase family member 11	UNIQUE			
	P13803	Electron transfer flavoprotein subunit alpha mitochondrial	UP	UNIQUE	DOWN	
	Q68FU3	Electron transfer flavoprotein subunit beta	UP	UP	DOWN	
	Q6UPE1	Electron transfer flavoprotein-ubiquinone oxidoreductase_ mitochondrial	UP	UP	DOWN	
	Q64591	2_4-dienoyl-CoA reductase_ mitochondrial	UP	UP	DOWN	
	P23965	Enoyl-CoA delta isomerase 1_ mitochondrial	UP	UP		
	Q5M884	Enoyl-CoA delta isomerase 2_ mitochondrial	UNIQUE		DOWN	
	P14604	Enoyl-CoA hydratase_ mitochondrial		UP	DOWN	
	O70351	3-hydroxyacyl-CoA dehydrogenase type-2	UP	UNIQUE		
	Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase mitochondrial	UP			
	P13437	3-ketoacyl-CoA thiolase_ mitochondrial	UP	UP	DOWN	UP
	P17764	Acetyl-CoA acetyltransferase_ mitochondrial	UP	UP	DOWN	
	P22791	Hydroxymethylglutaryl-CoA synthase_ mitochondrial	UP	UP	DOWN	UP
	P97519	Hydroxymethylglutaryl-CoA lyase_ mitochondrial	UNIQUE		UNIQUE	
	A0A0G2JSH2	3-hydroxybutyrate dehydrogenase type1 isoform CRA_a	UP	UP	DOWN	
TCA Cycle	B2GV15	Dihydrolipoamide acetyltransferase (E2)			UNIQUE	
	Q6P6R2	Dihydrolipoyl dehydrogenase_ mitochondrial (E3)	UNIQUE		UNIQUE	
	Q9ER34	Aconitate hydratase_ mitochondrial		UNIQUE		
	P56574	Isocitrate dehydrogenase [NADP]_ mitochondrial		UNIQUE		

	P13086	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha_mitochondrial		UP	UNIQUE	
	F1LPV8	Succinate--CoA ligase [GDP-forming] subunit beta_mitochondrial		UNIQUE	UNIQUE	
	P04636	Malate dehydrogenase_mitochondrial	UP	UP	DOWN	UP
	O88989	Malate dehydrogenase_cytoplasmic	UNIQUE	UNIQUE	UNIQUE	
	P41562	Isocitrate dehydrogenase [NADP] cytoplasmic	UNIQUE			
Electron Transport Chain	B2RZD6	NDUFA4_mitochondrial complex-associated	UP	UP	DOWN	UP
	P20070	NADH-cytochrome b5 reductase 3	UP	UP	DOWN	UP
	Q561S0	NADH dehydrogenase ubiquinone 1 alpha subcomplex subunit 10 mitochondrial		UP	DOWN	UP
	Q5BK63	NADH dehydrogenase ubiquinone 1 alpha subcomplex subunit 9 mitochondrial	UP		UNIQUE	
	P19234	NADH dehydrogenase ubiquinone flavoprotein 2 mitochondrial	UP	UNIQUE	UNIQUE	
	Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit mitochondrial	UP	UNIQUE	UNIQUE	UP
	Q641Y2	NADH dehydrogenase ubiquinone iron-sulfur protein 2 mitochondrial	UP	UP		
	Q920L2	Succinate dehydrogenase ubiquinone flavoprotein subunit mitochondrial		UNIQUE	UNIQUE	
	P21913	Succinate dehydrogenase ubiquinone iron-sulfur subunit mitochondrial	UP	UP	DOWN	
	Q5M9I5	Cytochrome b-c1 complex subunit 6_mitochondrial	UNIQUE	UNIQUE		
	Q68FY0	Cytochrome b-c1 complex subunit 1_mitochondrial	UP	UP	DOWN	
	P32551	Cytochrome b-c1 complex subunit 2_mitochondrial	UP	UP		UNIQUE
	P20788	Cytochrome b-c1 complex subunit Rieske mitochondrial	UP	UP		
	D3ZFAQ8	Cytochrome c-1		UP	DOWN	
	D3ZD09	Cytochrome c oxidase subunit	UP			UP

P00406	Cytochrome c oxidase subunit 2	UP	UP		UP
P10888	Cytochrome c oxidase subunit 4 isoform 1 mitochondrial	UP	UP	DOWN	
P11240	Cytochrome c oxidase subunit 5A_ mitochondrial	UP	UP	DOWN	DOWN
P10818	Cytochrome c oxidase subunit 6A1_ mitochondrial	UP	UP		
P11950	Cytochrome c oxidase subunit 6C-1		UP		
P11951	Cytochrome c oxidase subunit 6C-2		UP	DOWN	
P12075	Cytochrome c oxidase subunit 5B_ mitochondrial		UNIQUE		
P35171	Cytochrome c oxidase subunit 7A2_ mitochondrial		UNIQUE		
D3ZSB0	Cytochrome c oxidase subunit 6B1	UP	UP		DOWN
P62898	Cytochrome c_ somatic	UP	UNIQUE		
P15999	ATP synthase subunit alpha_ mitochondrial	UP	UP	DOWN	DOWN
P10719	ATP synthase subunit beta_ mitochondrial	UP	UP	DOWN	DOWN
P35434	ATP synthase subunit delta_ mitochondrial	UNIQUE	UP		
P31399	ATP synthase subunit d_ mitochondrial	UP	UP	DOWN	
P29419	ATP synthase subunit e_ mitochondrial	UNIQUE	UP	UNIQUE	
Q06647	ATP synthase subunit O_ mitochondrial		UP	DOWN	
P35435	ATP synthase subunit gamma_ mitochondrial	DOWN	UNIQUE	DOWN	
P21571	ATP synthase-coupling factor 6_ mitochondrial		UP		UNIQUE
P19511	ATP synthase F(0) complex subunit B1_ mitochondrial	UP	UNIQUE	DOWN	

Expression difference is indicated by DOWN, when the protein is down-regulated and by UP when the protein is up-regulated in the respective comparison. UNIQUE refers to proteins identified in only of the groups for each comparison. * Identification is based on protein identification from the UniProt protein database (<http://www.uniprot.org/>).

Table2. Proteins related to antioxidant system and calcium homeostasis that presented significantly altered expression in the liver mitochondria of rats treated with 0 (control), 15 or 50 mgF/L for 20 or 60 days.

	*Access number	Protein name description	20 days		60 days		20 days		60 days	
			Control	15mgF/L	Control	15mgF/L	Control	50mgF/L	Control	50mgF/L
Antioxidant system	B0BN47	Glutathione S-transferase	UNIQUE				UNIQUE			
	P04041	Glutathione peroxidase 1		DOWN		UNIQUE		DOWN		
	P00502	Glutathione S-transferase alpha-1		DOWN		UNIQUE		DOWN		
	P04903	Glutathione S-transferase alpha-2		DOWN		UNIQUE		DOWN		
	P04904	Glutathione S-transferase alpha-3		DOWN		UNIQUE		DOWN		
	P14942	Glutathione S-transferase alpha-4	UNIQUE					DOWN		
	P46418	Glutathione S-transferase alpha-5		DOWN		UNIQUE		DOWN		
	Q6AXY0	Glutathione S-transferase A6	UNIQUE			UNIQUE		DOWN		
	P24473	Glutathione S-transferase kappa 1		UP		UNIQUE	UNIQUE			
	P04905	Glutathione S-transferase Mu 1		UP		UNIQUE	UNIQUE			
	P08010	Glutathione S-transferase Mu 2				UNIQUE	UNIQUE			
	Q9Z1B2	Glutathione S-transferase Mu 5				UNIQUE				
	P08009	Glutathione S-transferase Yb-3				UNIQUE	UNIQUE			
	D3ZVQ8	Glutathione S-transferase_ mu 6-like				UNIQUE				
	P04762	Catalase				UP		DOWN		
	Q63716	Peroxiredoxin-1		DOWN		UNIQUE		DOWN		
	Q9Z0V6	Peroxiredoxin 3				UNIQUE				
	Q9Z0V5	Peroxiredoxin-4		DOWN		UNIQUE	UNIQUE			
	Q9R063	Peroxiredoxin-5_ mitochondrial		UNIQUE		UNIQUE				
	O35244	Peroxiredoxin-6	UNIQUE				UNIQUE			
	P67779	Prohibitin		UP						DOWN
	Q5XIH7	Prohibitin-2					DOWN			DOWN
	P07895	Superoxide dismutase [Mn]_ mitochondrial		UNIQUE		UNIQUE				

	P07632	Superoxide dismutase [Cu-Zn]			DOWN	DOWN
	P0DP29	Calmodulin-1		UNIQUE		UNIQUE
	P0DP30	Calmodulin-2	UP	UNIQUE		UNIQUE
	P0DP31	Calmodulin-3	UP	UNIQUE		UNIQUE
Calcium Homeostasis	M0RDI5	Mitochondrial calcium uniporter	UNIQUE		UNIQUE	
	Q5XIN6	Mitochondrial proton/calcium exchanger protein		UP		
	Q9Z2L0	Voltage-dependent anion-selective channel protein 1	UP	UP		
	P81155	Voltage-dependent anion-selective channel protein 2	UP	UP		
	Q9R1Z0	Voltage-dependent anion-selective channel protein 3	UP	UNIQUE		

Expression difference is indicated by DOWN, when the protein is down-regulated and by UP when the protein is up-regulated in the respective comparison. UNIQUE refers to proteins identified in only one of the groups for each comparison. * Identification is based on protein identification from the UniProt protein database (<http://www.uniprot.org/>).

DISCUSSION

In a recent study, we evaluated the effect of different F concentrations and exposure times on the profile of protein expression in the liver of rats. The most profound alterations were found in oxidative phosphorylation (OXPHOS), suggesting alterations in mitochondrial activity and energy metabolism (Pereira et al., 2018). Due to this, in the present study, we decided to evaluate mitochondria instead of whole liver, in order to better focus on the alterations induced by F in energy metabolism. Despite most of the identified proteins are involved in tricarboxylic acids (TCA) cycle and OXPHOS, some cytosolic proteins, mainly involved in glycolysis and gluconeogenesis, were also identified. This suggests that our sample, despite enriched in mitochondrial fraction, was not completely devoid of cytosolic proteins, which is commonly reported in other studies (Chae et al., 2018; Li et al., 2014; Morgenstern et al., 2017), and reflects the multi functionality of these proteins (Li et al., 2014).

Since our main aim was to identify changes in the proteins involved in energy metabolism, the alterations found in proteins related to glycolysis, gluconeogenesis, urea cycle, β -oxidation, TCA cycle and OXPHOS will be discussed separately. Considering that the energy metabolism is directly related to the production of ROS, alterations in antioxidant proteins will be also discussed, as well as calcium homeostasis.

For the bioinformatics analysis, most of the proteins with altered expression interacted with partners involved in signaling pathways, such as 5'-AMP-activated protein kinase catalytic subunit alpha-1 (Prkaa1- catalytic subunit), 5'-AMP-activated protein kinase subunit beta 1 (Prkab1- non catalytic subunit) and Mitogen-activated protein kinase 3 (MAPK3). PKaa1 and Pkab1 play an essential role in the regulation of energy metabolism. Upon decreased intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes (Cambridge et al., 2017; Foretz et al., 2010; Wang et al., 2010) (UNIPROT). On the other hand, MAPK3 has the function of modulating connections between

the extracellular signaling pathways, thus controlling cascades of apoptosis, growth and proliferation (Dhillon et al., 2007), which is also influenced by F (Otsuki et al., 2005).

Glycolysis and Gluconeogenesis

Many of the proteins with altered expression are related to glycolysis and gluconeogenesis (Table 1). Among them are enzymes participating in both pathways that were reduced upon exposure to F (both concentrations) in the short-term, such as Fructose-bisphosphate aldose B (ALDOB), Glyceraldehyde 3 phosphate dehydrogenase (GAPDH), Phosphoglycerate Kinase 1 (PGK1), Alpha enolase and two isoforms of L-lactate dehydrogenase (LDH) (B and C chain). In the present study, however, we found that F reduced many glycolytic enzymes, which, associated with the ability of this ion to also inhibit some of these enzymes explains the effects of this ion in the energy metabolism (Pereira et al., 2018).

It is important to highlight that upon exposure to the lowest F concentration in the short-term, there was an increase in L-lactate dehydrogenase A chain (LDHA) (Figure 3B; Table 1). Also LDHA was identified exclusively in the groups treated with 15 mgF/L (Figure 5A) and 50mg F/L for 60 days, when compared with their respective controls. This trend of increase in LDHA might represent an attempt to find alternative routes for energy production (Kotoh et al., 2011), as a consequence of glycolysis reduction, which has been reported in other studies (Dionizio et al., 2018; Malvezzi et al., 2018).

The impairment in glycolysis in the presence of F is expected to activate alternative pathways for glucose production from non-carbohydrate precursors, such as gluconeogenesis (Nelson and Cox, 2012). In our study, two major proteins related to gluconeogenesis, Pyruvate Carboxylase (PC) and Fructose 1,6 Bisphosphatase (FBP1), had their expressions changed in the F-treated groups. When the lowest dose of F was given in the short term, the enzyme PC was upregulated, while FBP1 was reduced upon exposure to F, regardless the dose. PC also plays an important anaplerotic role in order to replenish the intermediate compounds in TCA

cycle (Jitrapakdee et al., 2008; Jitrapakdee et al., 2006). This indicates that glucose synthesis through gluconeogenesis might not be occurring and highlights the anaplerotic role of PC upon exposure to F.

It is noteworthy that in the shorter period, many proteins related to glycolysis and gluconeogenesis were reduced, while in the longer period, they were increased (Table 1), which might be related to cell adaptation to the presence of F (Dabrowska et al. 2006; Pereira et al. 2018), allowing the maintenance of the energy flow. These findings highlight the importance of considering not only the dose, but also the time of exposure to F when interpreting studies conducted under different methodological designs.

β-Oxidation

Mitochondrial β-oxidation is a catabolic pathway with the participation of several enzymes, where the oxidation of fatty acids (FAO) generates Acetyl-CoA, which in turn can be completely degraded through the TCA cycle and the OXPHOS, to supply energy to different tissues when glycogen stores are scarce and energy demand remains high (Ghimire and Dhamoon, 2018; Leone et al., 1999; Vishwanath, 2016; Wanders et al., 2010). In the present study, several enzymes involved in different steps of FAO had change in expression that was distinct dependent on the dose of F and time of treatment. In general, the enzymes involved in β-oxidation were upregulated upon exposure to F, except for the dose of 50 mgF/L administered for 20 days that led to downregulation (Table 1). Two important enzymes involved in the transport of fatty acids were altered. Carnitine Palmitoyltransferase (CPT1) was absent in the group that received 50 mgF/L for 20 days, while mitochondrial carnitine/acylcarnitine carrier (CACT) was exclusively found in the groups treated with F for 20 days and at the group treated with the highest dose for 60 days. The presence of these transporters in the groups treated with F seems to be an attempt to keep the availability of fatty acids to be oxidized in order to maintain the energy flow (Vishwanath, 2016).

On the other hand, enzymes involved in the production of ketone bodies were also altered. We observed an increase of the enzyme Hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase) in the groups that received F, regardless of the period (except for the 50 mgF/L group treated for 20 days), while Hydroxymethylglutaryl-CoA lyase (HMG-CoA lyase) was absent in the groups treated with F for 20 days. These results indicate that the production of acetoacetate might have been increased in the groups treated with F for 60 days, due to the increase in HMG-CoA synthase, one of the enzymes that limit the speed of the ketogenic pathway (Dashti and Ontko, 1979).

Urea Cycle

The urea cycle is an essential route for the excretion of nitrogen from the organism, occurring initially in the hepatic mitochondria and ending in the cytosol. When excessive amounts of amino acids are present and cannot be stored, they are therefore degraded (Devlin, 2011). Long-term changes in the activities of urea cycle enzymes may occur as response to alterations in the nitrogen flux of amino acids caused by changes in protein consumption, catabolism of endogenous proteins or when amino acids are diverted to anaplerotic reactions to be used in other energy-generating pathways (Morris, 2002).

Our results revealed that Mitochondrial glutamate dehydrogenase (GLUD1) and Mitochondrial aspartate aminotransferase, also known as Aspartate transaminase (AST) showed increased expression or were exclusive in both periods and doses of F administered (except for the group that received the highest dose of F for 20 days; Table 1), compared to the control group. This increased expression might be an attempt to keep the catabolism of amino acids, since it has been reported that exposure to F (10 and 30 mgF/L in the drinking water for 50 days) inhibited the activity of AST and Glutamate dehydrogenase (Grucka-Mamczar E, 1997).

Regarding the enzymes involved in the urea cycle, Carbamoyl-phosphate synthase ammonia mitochondrial (CPS1) and Ornithine carbamoyltransferase_mitochondrial (OTC), respectively first and second enzymes of the cycle were increased upon exposure to 15 mgF/L for 20 days, but decreased upon exposure to the highest F dose for the same period. Their increase upon exposure to the lowest dose might be an attempt to keep the urea cycle flow due to the reduction in downstream enzymes. Among these, Argininosuccinate synthase (ASS1) was reduced in the groups treated for 20 days and exclusive in the groups treated for 60 days, regardless the dose of F (Table 1). Reduction in the expression of ASS1 can increase cellular aspartate and this amino acid can be anaplerotically diverted to the TCA cycle in order to maintain the energy flux (Kremer et al., 2017). This is consistent with the reduction in several energetic pathways and also with the increase in Malate dehydrogenase_mitochondrial (MDH2) upon exposure to F (regardless the dose) in the short-term. The anaplerotic conversion of aspartate to oxaloacetate (OAA), coupled to increase in MDH2, can be an attempt to increase the rate of the TCA cycle in the short-term. Consistent with the reduction in ASS1 in the short-period, there was also reduction in Arginase 1 (ARG1), regardless the dose of F (Table 1), as has been reported in the literature (Pethe et al., 2002; Van Pilsum et al., 1970). The decrease in ARG1, the last enzyme of the urea cycle, is consistent with the downregulation of upstream enzymes, indicating reduced flow of the cycle.

Tricarboxylic Acid Cycle

The TCA cycle is an important pathway of catabolic and anabolic reactions. The cycle generates two important products, the reductive compounds, NADH and FADH₂, which contain a large amount of energy stored in their molecules that will be oxidized in the ETC to generate ATP (Devlin, 2011; Martinez-Reyes et al., 2016; Nelson and Cox, 2012).

The most relevant finding involving TCA cycle enzymes was the increase in MDH2 upon exposure to F, except for the dose of 50 mgF/L administered for 20 days that led to

downregulation of this enzyme (Table 1). MDH2 is a key enzyme in the TCA cycle, since one of its functions is the interconversion of malate to OAA using the NAD/NADH coenzyme system. In addition, its activity is essential to control the redox system of the mitochondrial matrix, through its action in the regulation of TCA cycle (Devlin, 2011; Goward and Nicholls, 1994; Linden et al., 2016; Nunes-Nesi et al., 2005). Moreover, the inhibition of the mitochondrial ETC is related to the inhibition of the activity of MDH2, since its cofactor is essential for the mitochondrial complexes and this inhibition can disturb mitochondrial respiration (Ban et al., 2016). Thus, increase in this enzyme upon exposure to F is consistent with the increase in all energetic pathways observed in the present study, since TCA cycle connects all pathways involved in energy generation.

Oxidative phosphorylation

In our study, chronic administration of F produced significant changes in the OXPHOS. For the lowest dose of F, regardless the period of treatment, mitochondrial complexes I, II, III, IV and V had their expression increased or were exclusive in the treated groups (except for the ATP synthase subunit gamma mitochondrial that was reduced in the period of 20 days). On the other hand, upon exposure to the highest dose of F in the short period, the proteins were reduced or absent (except for the ATP synthase subunit e mitochondrial that was exclusively upon exposure to F), which was also observed in other energy pathways (Table 1). On the other hand, when administered the dose of 50 mgF/L for 60 days, the enzymes of complex I were increased, those of complex II were absent, the ones of complex III were reduced, and for complex IV, two isoforms of cytochrome oxidase were increased and two were reduced. A reduction was also observed for the complex V.

Studies investigating the effects of F in energy metabolism have shown a decrease in expression or inhibition of the activity of the mitochondrial complexes when F is administered (Chauhan et al., 2013; Fina et al., 2014; He et al., 2012; Liang et al., 2016; Panneerselvam et

al., 2017; Wang et al., 2017). According to Fina et al. (2014), chronic or acute exposure to F in vitro inhibits complexes I and III of the OXPHOS, which may result in an increase in the release of superoxide anions, thus increasing ROS levels.

An important enzyme that was altered upon exposure to F was Cytochrome c-1 (CYC1) (Table 1). This enzyme is part of the complex III and is considered the major pathway for free radical generation within mitochondria. It regulates the mitochondrial function, besides being an important link between electron transport chain and ATP production (Cela et al., 2010; Roy Chowdhury and Banerji, 2018; Shimada et al., 2018). Mitochondria can sense signs of metabolic damage caused by increase in ROS generation and compromise cells to apoptosis with the release of death-inducing factors, such as Cyt c in the cytosol due to permeabilization of the mitochondrial outer membrane (Zhu et al., 2012). However, in the present study, increased production of ROS that might have occurred in the groups treated with F (except for 50 mgF/L for 20 days) might have been counteracted by increase in the expression of antioxidant enzymes and antiapoptotic proteins, as will be discussed below.

Antioxidant system and Calcium homeostasis

In the present study, in general several isoforms of Superoxide dismutase (SOD), Prohibitin (PHB), Glutathione peroxidase (GPx), Peroxiredoxin (PRX) and Catalase (CAT) were reduced or absent when F was administered for 20 days, regardless the dose (Table 2, Tables S1-S4). Nevertheless, when F was administered for the longer period, most of these enzymes were increased or were exclusively identified in the group treated with 15 mgF/L, which indicates an attempt to counteract the generation of ROS due to the vigorous energy metabolism. Interestingly, exposure to 50 mgF/L for 20 days led to a reduction in energy metabolism, with probably low ROS generation, what in turn led to reduction in antioxidant enzymes (Tables 1 and 2). Despite of that, in the long term, an increase in the energy

metabolism was observed for the 50 mgF/L group, but most of the antioxidant enzymes did not present change in expression.

Another reason for ROS generation is the accumulation or excessive influx of calcium (Ca^{2+}) into the mitochondria (K., 2018). Normal levels of Ca^{2+} within the mitochondria can activate several dehydrogenase enzymes, thus raising the level of respiratory rate as well as proton extrusion and ATP production. On the other hand, Ca^{2+} overload may impair mitochondrial homeostasis, increase ROS and cause changes in the mitochondrial membrane potential, besides increasing the opening of the mitochondrial permeability transition pore (MPTP) complex, resulting in apoptosis through the release of Cyt c (Demaurex and Distelhorst, 2003; Giorgi et al., 2008; Kirichok et al., 2004; McCormack and Denton, 1989; Pereira et al., 2018; Santo-Domingo and Demareux, 2010).

Some proteins that are involved in calcium homeostasis and apoptosis were altered upon exposure to F, such as: 1) isoforms of Voltage-dependent anion-selective channel protein (VDAC) that were increased in the lowest dose of F regardless the period; 2) isoforms of Calmodulin that were increased or exclusively identified in the treated groups in both periods (except for the highest dose of F for 20 days); 3) Mitochondrial proton/calcium exchanger that was increased in the lowest dose of F for 60 days; 4) Mitochondrial calcium uniporter (mCU) was identified exclusively in the control groups; and 5) 3-ketoacyl-CoA thiolase_mitochondrial that was increased in all groups except in the group treated with 50 mgF/L for 20 days (Tables 1 and 2).

Our results regarding the proteins related to Ca^{2+} homeostasis and apoptosis that were altered upon exposure to F indicate an attempt to maintain Ca^{2+} homeostasis and avoid apoptosis. In this sense, the increase in Calmodulin could help to inactivate mCU activity, thus preventing Ca^{2+} accumulation in the mitochondria (Moreau et al., 2006; Santo-Domingo and Demareux, 2010). On the other hand, VDAC may participate in the formation of the MPTP

complex, responsible for the release of mitochondrial products that triggers apoptosis (UNIPROT). The increase in VDAC was counteracted by the increase in 3-ketoacyl-CoA thiolase_mitochondrial, an enzyme that links fatty acid metabolism to apoptosis, since it abolishes cellular protein Bcl-2/adenovirus E1B 19 kD-interacting protein 3 (BNIP3)-mediated apoptosis by blocking mitochondrial damage (Cao et al., 2008). Mitochondrial damage may result in an energetic catastrophe due to disruption of electron transport and ATP production (Green and Reed, 1998) and lead to apoptosis (Lamy et al., 2003). BNIP3 may initiate mitochondria-mediated apoptosis by inducing MPTP opening to disrupt the proton electrochemical gradient across the inner mitochondrial membrane (Kubli et al., 2007). The result is reduced mitochondrial respiration, excess generation of ROS and reduced ATP synthesis that can lead to cell death (Vande Velde et al., 2000). Thus, increase in 3-ketoacyl-CoA thiolase_mitochondrial in the groups that were having vigorous energy flux might have prevented opening of the MPTP that could lead to apoptosis.

In summary, our results indicate that the direction of the energy flux depends on the dose of F and time of exposure. The dose of 15 mgF/L, when administered for 20 days, leads to a reduction in glycolysis, which is counteracted by an increase in β -oxidation, urea cycle and OXPHOS. At 60 days, however, an increase in all energetic pathways occurs. To compensate for the generation of ROS due to the vigorous state of energy production, there is a concomitant increase in antioxidant enzymes and in antiapoptotic proteins. However, the dose of 50 mgF/L, when administered for 20 days, reduces the enzymes involved in all energetic pathways. With less energy production there is probably less generation of ROS, what leads to reduction also in antioxidant enzymes. However, when 50 mgF/L is administered for 60 days, an increase in energy metabolism is observed, but the antioxidant enzymes in general do not present change in expression. Additional studies should be conducted employing longer periods of administration of high F doses, in order that the relationship between energy production and

oxidative balance can be better understood. When analyzed in conjunction, these results suggest that the organism seems to adapt to the effects of F along time, activating pathways to reduce the toxicity of this ion. Ultimately, our findings corroborate the safety of the use of F for caries control.

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Conflict of interest statement

The authors have declared no conflict of interest.

Author's contribution

Araujo TT, Pereira HABS and Buzalaf MAR conceived the experiments. Araujo TT, Pereira HABS, Dionizio A, Sanches CC and Fernandes MS conducted the experiments. Araujo TT, Dionizio A and Carvalho T tabulated the data. Araujo TT, Dionizio A, Pereira HABS and Buzalaf MAR analyzed and interpreted the results. Araujo TT and Buzalaf MAR drafted the article. All authors reviewed and approved the manuscript.

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Supplementary material

Supplementary table 1. Proteins with expression significantly altered in the mitochondria of liver of rats of group treated with 15 mgF/L for 20 days x control group comparison.

^a Accession number	Protein name	PLGS Score	^b Ratio 15 mgF/L: Control
A0A0G2K8W9	Spectrin beta chain	43	12.81
F1LX07	Solute carrier family 25 member 12	62	3.39
Q7TP12	Cc2-17	71	2.64
B0BMT9	Sqrdl protein	82	2.61
Q561S0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10_ mitochondrial	402	2.51
F1M609	Acyl-coenzyme A oxidase	17	2.10
B1H250	Glycine-N-acyltransferase-like 1	88	2.03
M0R6N2	MOSC domain-containing protein 2_ mitochondrial-like	156	1.95
O88994	Mitochondrial amidoxime reducing component 2	154	1.88
P02262	Histone H2A type 1	8057	1.82
Q64598	Histone H2A type 1-F	8057	1.82
P0C169	Histone H2A type 1-C	8057	1.80
P0C170	Histone H2A type 1-E	8057	1.80
P0CC09	Histone H2A type 2-A	8057	1.80
Q4FZT6	Histone H2A type 3	8057	1.80
Q00728	Histone H2A type 4	8057	1.80
P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2_ mitochondrial	127	1.79
P04799	Cytochrome P450 1A2	208	1.70
P24473	Glutathione S-transferase kappa 1	206	1.70
P32089	Tricarboxylate transport protein_ mitochondrial	241	1.68
F1LQ55	Podocan	54	1.65
B2RYS2	Cytochrome b-c1 complex subunit 7	176	1.62
P11915	Non-specific lipid-transfer protein	54	1.52
P00406	Cytochrome c oxidase subunit 2	562	1.51
D3ZSB0	Cytochrome c oxidase subunit 6B1	485	1.49
A0A0G2JSH2	3-hydroxybutyrate dehydrogenase_ type 1_ isoform CRA_a	421	1.48
P05182	Cytochrome P450 2E1	391	1.48
P29147	D-beta-hydroxybutyrate dehydrogenase_ mitochondrial	421	1.48
A0A0G2K7R1	Uncharacterized protein	7927	1.48
Q9R1Z0	Voltage-dependent anion-selective channel protein 3	253	1.48
P17764	Acetyl-CoA acetyltransferase_ mitochondrial	476	1.46
D4ABV5	Calmodulin-2	170	1.46
D3ZD09	Cytochrome c oxidase subunit	485	1.46
Q64428	Trifunctional enzyme subunit alpha_ mitochondrial	593	1.46
A0A0G2K459	Mitochondrial carrier 2	584	1.45
Q9Z2L0	Voltage-dependent anion-selective channel protein 1	273	1.45
Q5U206	Calmodulin-like protein 3	67	1.43
D4A5L9	Similar to Cytochrome c_ somatic	283	1.40
Q09073	ADP/ATP translocase 2	490	1.39
P19643	Amine oxidase [flavin-containing] B	142	1.39

P32551	Cytochrome b-c1 complex subunit 2_ mitochondrial	188	1.39
P10818	Cytochrome c oxidase subunit 6A1_ mitochondrial	737	1.39
P04905	Glutathione S-transferase Mu 1	208	1.39
Q5BK63	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9_ mitochondrial	247	1.39
P15999	ATP synthase subunit alpha_ mitochondrial	7255	1.38
Q68FY0	Cytochrome b-c1 complex subunit 1_ mitochondrial	683	1.38
A0A0G2K099	Uncharacterized protein	2416	1.38
P25235	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	76	1.34
P22791	Hydroxymethylglutaryl-CoA synthase_ mitochondrial	849	1.34
O70351	3-hydroxyacyl-CoA dehydrogenase type-2	149	1.32
Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase_ mitochondrial	725	1.32
O89035	Mitochondrial dicarboxylate carrier	406	1.32
B2RZD6	NDUFA4_ mitochondrial complex-associated	1982	1.32
Q64591	2_4-dienoyl-CoA reductase_ mitochondrial	461	1.31
P16970	ATP-binding cassette sub-family D member 3	163	1.31
P62898	Cytochrome c_ somatic	283	1.31
Q68G44	Hydroxymethylglutaryl-CoA synthase	849	1.31
Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit_ mitochondrial	84	1.31
Q60587	Trifunctional enzyme subunit beta_ mitochondrial	378	1.30
P10860	Glutamate dehydrogenase 1_ mitochondrial	1100	1.28
P04636	Malate dehydrogenase_ mitochondrial	1868	1.28
P11240	Cytochrome c oxidase subunit 5A_ mitochondrial	1638	1.27
P05178	Cytochrome P450 2C6	459	1.27
P13803	Electron transfer flavoprotein subunit alpha_ mitochondrial	572	1.27
P67779	Prohibitin	1623	1.27
F1LZW6	Solute carrier family 25 member 13	350	1.27
P19511	ATP synthase F(0) complex subunit B1_ mitochondrial	279	1.26
D3ZFH6	Prohibitin_ pseudogene 1	280	1.26
Q6UPE0	Choline dehydrogenase_ mitochondrial	1051	1.23
P13437	3-ketoacyl-CoA thiolase_ mitochondrial	679	1.22
P20788	Cytochrome b-c1 complex subunit Rieske_ mitochondrial	1112	1.22
Q6IN39	Hsd17b4 protein	295	1.22
Q05962	ADP/ATP translocase 1	383	1.21
P00481	Ornithine carbamoyltransferase_ mitochondrial	760	1.21
P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit_ mitochondrial	119	1.21
P81155	Voltage-dependent anion-selective channel protein 2	263	1.21
G3V9Z3	Amine oxidase	212	1.20
Q9JM53	Apoptosis-inducing factor 1_ mitochondrial	161	1.20
M0RB90	Cytochrome P450_ family 2_ subfamily C_ polypeptide 6_ variant 1	459	1.20
P0C0S7	Histone H2A.Z	4377	1.20
P24329	Thiosulfate sulfurtransferase	1235	1.20
P31399	ATP synthase subunit d_ mitochondrial	850	1.19
Q68FU3	Electron transfer flavoprotein subunit beta	559	1.19
D4AEC0	Histone H2A	4377	1.19

P18163	Long-chain-fatty-acid--CoA ligase 1	2897	1.19
Q5XIH7	Prohibitin-2	1071	1.19
P11884	Aldehyde dehydrogenase_ mitochondrial	324	1.17
P10888	Cytochrome c oxidase subunit 4 isoform 1_ mitochondrial	2005	1.17
Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_ mitochondrial	336	1.17
P21396	Amine oxidase [flavin-containing] A	212	1.16
A0A0G2K193	Cytochrome P450 2A2	79	1.16
Q6UPE1	Electron transfer flavoprotein-ubiquinone oxidoreductase_ mitochondrial	373	1.16
G3V6I4	Mitochondrial amidoxime reducing component 1	272	1.16
P11711	Cytochrome P450 2A1	122	1.14
A0A0G2K8G2	Acyl-CoA synthetase medium-chain family member 1	227	1.13
P00507	Aspartate aminotransferase_ mitochondrial	547	1.13
G3V7J5	Carboxylic ester hydrolase	50	1.13
P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	327	1.13
P30839	Fatty aldehyde dehydrogenase	163	1.13
Q68G41	Dodecenoyl-Coenzyme A delta isomerase (3_2 trans-enoyl-Coenzyme A isomerase)	540	1.12
P23965	Enoyl-CoA delta isomerase 1_ mitochondrial	540	1.12
Q6IRK9	Carboxypeptidase Q	204	1.11
P04642	L-lactate dehydrogenase A chain	212	1.11
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating]_ mitochondrial	482	1.11
G3V9W6	Aldehyde dehydrogenase	133	1.09
G3V7J0	Aldehyde dehydrogenase family 6_ subfamily A1_ isoform CRA_b	482	1.09
P10719	ATP synthase subunit beta_ mitochondrial	9561	1.09
P52873	Pyruvate carboxylase_ mitochondrial	67	1.09
P97524	Very long-chain acyl-CoA synthetase	154	1.09
P07756	Carbamoyl-phosphate synthase [ammonia]_ mitochondrial	3247	1.07
Q62812	Myosin-9	337	1.04
Q5BJZ3	Nicotinamide nucleotide transhydrogenase	158	0.90
P10633	Cytochrome P450 2D1	425	0.89
F1LNF0	Myosin heavy chain 14	31	0.89
P00388	NADPH--cytochrome P450 reductase	119	0.89
A0A0G2K1P0	Peptidyl-prolyl cis-trans isomerase	1120	0.89
P10111	Peptidyl-prolyl cis-trans isomerase A	1120	0.89
P46418	Glutathione S-transferase alpha-5	418	0.88
P15865	Histone H1.4	406	0.88
G3V9C7	Histone H2B	9537	0.88
P07824	Arginase-1	6511	0.87
P12939	Cytochrome P450 2D10	425	0.87
Q5I0P9	Cytochrome P450_ family 2_ subfamily d_ polypeptide 5	409	0.87
P06685	Sodium/potassium-transporting ATPase subunit alpha-1	459	0.87
P60711	Actin_ cytoplasmic 1	8996	0.86
P63259	Actin_ cytoplasmic 2	8996	0.86
Q64680	Cytochrome P450 2D4	251	0.86
P80254	D-dopachrome decarboxylase	495	0.86

Q66HT1	Fructose-bisphosphate aldolase	2633	0.86
P00884	Fructose-bisphosphate aldolase B	2481	0.85
B2GV99	Myl6 protein	1733	0.85
Q64119	Myosin light polypeptide 6	1733	0.85
P11598	Protein disulfide-isomerase A3	84	0.85
P14740	Dipeptidyl peptidase 4	87	0.84
P12336	Solute carrier family 2_ facilitated glucose transporter member 2	330	0.84
A0A0G2K926	Alpha-1-inhibitor III	65	0.83
P18757	Cystathionine gamma-lyase	366	0.83
P08683	Cytochrome P450 2C11	228	0.83
D3ZEN2	Uncharacterized protein	206	0.83
P11030	Acyl-CoA-binding protein	1361	0.82
Q498M9	Similar to glyceraldehyde-3-phosphate dehydrogenase	206	0.82
P06686	Sodium/potassium-transporting ATPase subunit alpha-2	255	0.82
A0A1W2Q605	Solute carrier family 25 member 31 (Fragment)	214	0.82
Q9EQS4	Cystathionase (Cystathionine gamma-lyase)	350	0.81
P63018	Heat shock cognate 71 kDa protein	104	0.81
P05183	Cytochrome P450 3A2	375	0.80
P08011	Microsomal glutathione S-transferase 1	169	0.80
M0RCB1	Uncharacterized protein	104	0.80
P06687	Sodium/potassium-transporting ATPase subunit alpha-3	245	0.79
P50137	Transketolase	133	0.79
P26453	Basigin	695	0.79
P25093	Fumarylacetoacetase	296	0.79
Q00729	Histone H2B type 1-A	2473	0.79
F1LZ34	S-adenosylmethionine synthase	104	0.79
P13444	S-adenosylmethionine synthase isoform type-1	104	0.79
P14659	Heat shock-related 70 kDa protein 2	137	0.78
P09626	Potassium-transporting ATPase alpha chain 1	32	0.78
P09118	Uricase	18072	0.77
M0R8T2	10-formyltetrahydrofolate dehydrogenase	106	0.77
P31210	3-oxo-5-beta-steroid 4-dehydrogenase	87	0.76
P06761	78 kDa glucose-regulated protein	455	0.76
Q03248	Beta-ureidopropionase	94	0.76
F1LRK1	Sodium/potassium-transporting ATPase subunit alpha	32	0.76
F1LZI1	Similar to heat shock protein 8	76	0.75
P35565	Calnexin	406	0.74
P00173	Cytochrome b5	5757	0.74
Q63010	Liver carboxylesterase B-1	86	0.74
A0A0G2K3Z9	Uncharacterized protein	318	0.74
P19112	Fructose-1_6-bisphosphatase 1	355	0.73
Q63716	Peroxiredoxin-1	334	0.73
P04785	Protein disulfide-isomerase	706	0.73
Q63108	Carboxylesterase 1E	71	0.73
M0R757	Elongation factor 1-alpha	1541	0.73
P62630	Elongation factor 1-alpha 1	1541	0.73
P04797	Glyceraldehyde-3-phosphate dehydrogenase	1509	0.73

P62804	Histone H4	15720	0.73
Q9JJ40	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	326	0.73
F1M6C2	Elongation factor 1-alpha 1 pseudogene	1541	0.72
P00502	Glutathione S-transferase alpha-1	977	0.72
P13107	Cytochrome P450 2B3	187	0.71
D3ZRN3	Actin_ beta-like 2	2843	0.70
Q9Z0V5	Peroxiredoxin-4	118	0.70
P62632	Elongation factor 1-alpha 2	727	0.69
P04903	Glutathione S-transferase alpha-2	1035	0.69
P18418	Calreticulin	605	0.68
Q64541	Sodium/potassium-transporting ATPase subunit alpha-4	90	0.68
P09034	Argininosuccinate synthase	247	0.68
P20816	Cytochrome P450 4A2	92	0.68
D3ZQT0	Uncharacterized protein	360	0.68
P20817	Cytochrome P450 4A14	224	0.67
Q63081	Protein disulfide-isomerase A6	251	0.67
P54708	Potassium-transporting ATPase alpha chain 2	68	0.66
P14141	Carbonic anhydrase 3	236	0.65
Q9JLJ3	4-trimethylaminobutyraldehyde dehydrogenase	100	0.64
Q4QRB4	Tubulin beta-3 chain	81	0.64
Q03336	Regucalcin	120	0.63
P04764	Alpha-enolase	130	0.63
Q6P9T8	Tubulin beta-4B chain	61	0.62
M0R5J4	Uncharacterized protein	130	0.62
O09171	Betaine--homocysteine S-methyltransferase 1	2501	0.61
B4F7C2	Tubulin beta chain	61	0.61
A0A0G2K9I5	Similar to H3 histone_ family 3B	1666	0.60
P52759	2-iminobutanoate/2-iminopropanoate deaminase	793	0.59
P04041	Glutathione peroxidase 1	568	0.59
P0DMW0	Heat shock 70 kDa protein 1A	198	0.59
P0DMW1	Heat shock 70 kDa protein 1B	198	0.59
P55063	Heat shock 70 kDa protein 1-like	186	0.59
P35435	ATP synthase subunit gamma_ mitochondrial	226	0.58
P84245	Histone H3.3	3274	0.58
Q6LED0	Histone H3.1	3178	0.58
B6RK61	Myosin heavy chain 7B	157	0.58
Q8VIF7	Selenium-binding protein 1	81	0.58
D3ZPY8	Aldo-keto reductase family 1_ member C13	89	0.57
Q5I0M4	Aldo-keto reductase family 1_ member C12	89	0.57
D3ZEL2	Aldo-keto reductase family 1_ member C19	89	0.57
P04462	Myosin-8 (Fragment)	638	0.53
P02564	Myosin-7	288	0.52
Q29RW1	Myosin-4	390	0.51
P04904	Glutathione S-transferase alpha-3	1837	0.50
P02563	Myosin-6	268	0.50
P85108	Tubulin beta-2A chain	105	0.50
P69897	Tubulin beta-5 chain	105	0.50
F1LRV9	Myosin heavy chain 1	1123	0.46

P12847	Myosin-3	209	0.46
P02600	Myosin light chain 1/3_ skeletal muscle isoform	859	0.44
F1LST0	HEAT repeat-containing 5B	55	0.37
O54753	17-beta-hydroxysteroid dehydrogenase type 6	41	15 mgF/L*
Q9Z1N4	3'(2')_5'-bisphosphate nucleotidase 1	78	15 mgF/L
Q7TPA1	Ab1-114	170	15 mgF/L
A0A0G2JW01	Actin-binding LIM protein 1	83	15 mgF/L
Q8R4I6	Actinin alpha 3	48	15 mgF/L
B3DMA2	Acyl-CoA dehydrogenase family member 11	113	15 mgF/L
Q66HA6	ADP-ribosylation factor-like protein 8B	175	15 mgF/L
F1M5N3	Ankyrin 2	21	15 mgF/L
Q5I0M1	Apolipoprotein H	285	15 mgF/L
B1WBP7	ATP synthase subunit delta_ mitochondrial	514	15 mgF/L
P29419	ATP synthase subunit e_ mitochondrial	1031	15 mgF/L
D3ZX04	ATP-binding cassette subfamily C member 10	52	15 mgF/L
A1L1L0	BBX_ HMG-box-containing	67	15 mgF/L
B0BN82	Brix domain containing 1	75	15 mgF/L
D3ZJJ4	Calpain 15	35	15 mgF/L
F1M5A1	Calreticulin 4	38	15 mgF/L
Q9QYJ6	cAMP and cAMP-inhibited cGMP 3'_5'-cyclic phosphodiesterase 10A	48	15 mgF/L
P10959	Carboxylesterase 1C	54	15 mgF/L
D3ZP14	Carboxylic ester hydrolase	31	15 mgF/L
Q6AYS3	Carboxypeptidase	100	15 mgF/L
B0K020	CDGSH iron-sulfur domain-containing protein 1	408	15 mgF/L
A0A0G2JXQ2	Chromodomain helicase DNA-binding protein 9	31	15 mgF/L
P15978	Class I histocompatibility antigen_ Non-RT1.A alpha-1 chain	46	15 mgF/L
Q5M9I5	Cytochrome b-c1 complex subunit 6_ mitochondrial	875	15 mgF/L
P00185	Cytochrome P450 1A1	40	15 mgF/L
F1LME8	Cytochrome P450 2B9-like	36	15 mgF/L
D3Z7Y1	Cytochrome P450 2C7	65	15 mgF/L
Q5RKL4	Dimethylglycine dehydrogenase	38	15 mgF/L
Q63342	Dimethylglycine dehydrogenase_ mitochondrial	38	15 mgF/L
Q924C3	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	60	15 mgF/L
Q6P7Q0	Electron transfer flavoprotein beta subunit lysine methyltransferase	74	15 mgF/L
P85834	Elongation factor Tu_ mitochondrial	32	15 mgF/L
Q62730	Estradiol 17-beta-dehydrogenase 2	101	15 mgF/L
Q66H61	Glutamine--tRNA ligase	38	15 mgF/L
M0RDR7	Glutamine--tRNA ligase-like	34	15 mgF/L
D3ZT90	Glutaryl-CoA dehydrogenase	63	15 mgF/L
Q5PQT3	Glycine N-acyltransferase	88	15 mgF/L
Q6HA10	Growth/differentiation factor 6	38	15 mgF/L
Q8K586	GTP-binding nuclear protein Ran_ testis-specific isoform	46	15 mgF/L
F7F2F3	Heat shock 70 kDa protein 4L	34	15 mgF/L
Q5XHZ0	Heat shock protein 75 kDa_ mitochondrial	42	15 mgF/L
P82995	Heat shock protein HSP 90-alpha	40	15 mgF/L

P34058	Heat shock protein HSP 90-beta	64	15 mgF/L
F1LZX5	HECT domain E3 ubiquitin protein ligase 4	33	15 mgF/L
A6YP92	Homeobox protein ARX	70	15 mgF/L
Q2V057	Hydroxyproline dehydrogenase	420	15 mgF/L
G3V8L7	Integrin alpha M	55	15 mgF/L
A0A0G2K4L8	Integrin subunit alpha M	55	15 mgF/L
G3V7Q7	IQ motif containing GTPase activating protein 1 (Predicted)_ isoform CRA_b	55	15 mgF/L
M0R8J3	Junction-mediating and regulatory protein	27	15 mgF/L
Q5XHZ6	Kelch-like protein 7	47	15 mgF/L
O88658	Kinesin-like protein KIF1B	28	15 mgF/L
O88867	Kynurenine 3-monooxygenase	152	15 mgF/L
Q62959	Leptin receptor	58	15 mgF/L
F1M9F2	Leptin receptor gene-related protein	53	15 mgF/L
D3ZWK4	Lethal(3)malignant brain tumor-like protein 1	34	15 mgF/L
F1LNJ1	Leucine-rich repeat kinase 2	31	15 mgF/L
Q6TXF5	Leucine-rich repeat-containing protein 66	48	15 mgF/L
P33124	Long-chain-fatty-acid--CoA ligase 6	27	15 mgF/L
Q9QYP1	Low-density lipoprotein receptor-related protein 4	20	15 mgF/L
Q5XIM7	Lysine--tRNA ligase	51	15 mgF/L
Q63341	Macrophage metalloelastase	129	15 mgF/L
P57113	Maleylacetoacetate isomerase	56	15 mgF/L
R9PXT7	Matrix metalloproteinase	129	15 mgF/L
Q9WVR8	Menin	49	15 mgF/L
Q3KR86	MICOS complex subunit Mic60 (Fragment)	256	15 mgF/L
P97521	Mitochondrial carnitine/acylcarnitine carrier protein	89	15 mgF/L
D4A833	Mitochondrial ribosomal protein S30	50	15 mgF/L
Q63538	Mitogen-activated protein kinase 12	54	15 mgF/L
Q9ERE6	Myosin phosphatase Rho-interacting protein	33	15 mgF/L
Q6AXQ4	Myotubularin	57	15 mgF/L
B2RYS8	NADH dehydrogenase [ubiquinone] 1 beta subcomplex subunit 8_ mitochondrial	222	15 mgF/L
P11661	NADH-ubiquinone oxidoreductase chain 5	63	15 mgF/L
A0A140UHX3	Non-SMC condensin II complex_ subunit D3	41	15 mgF/L
Q9ERC5	Otoferlin	42	15 mgF/L
D4A0F3	Oxysterol-binding protein	29	15 mgF/L
Q9R063	Peroxisomal protein 5_ mitochondrial	164	15 mgF/L
F1LM99	PHD finger protein 12	40	15 mgF/L
F1LX13	Phosphodiesterase	42	15 mgF/L
M0RDK4	PITPNM family member 3	68	15 mgF/L
Q9JID1	Programmed cell death protein 4	146	15 mgF/L
A0A0G2K5H0	Proline dehydrogenase	94	15 mgF/L
Q5XIN5	Protein FAM228A	68	15 mgF/L
D3ZG37	Protein phosphatase 6_ regulatory subunit 1	84	15 mgF/L
A0A0G2K8V0	Protein tyrosine phosphatase_ receptor type_ M	35	15 mgF/L
P01134	Protransforming growth factor alpha	155	15 mgF/L
A0A0H2UHN7	RCG24013_ isoform CRA_a	58	15 mgF/L
F1LRY5	RCG45398	50	15 mgF/L
Q5XIF2	RCG47444_ isoform CRA_a	86	15 mgF/L

D3Z9V1	RELB proto-oncogene_ NF-kB subunit	128	15 mgF/L
P16391	RT1 class I histocompatibility antigen_ AA alpha chain	66	15 mgF/L
P18298	S-adenosylmethionine synthase isoform type-2	39	15 mgF/L
Q64380	Sarcosine dehydrogenase_ mitochondrial	56	15 mgF/L
Q9JLS3	Serine/threonine-protein kinase TAO2	36	15 mgF/L
F1M7K9	Similar to elongation protein 4 homolog	70	15 mgF/L
D4A3C9	Small nuclear RNA-activating complex_ polypeptide 4	33	15 mgF/L
Q6IRK8	Spectrin alpha chain_ non-erythrocytic 1	40	15 mgF/L
P17178	Sterol 26-hydroxylase_ mitochondrial	58	15 mgF/L
P50237	Sulfotransferase 1C1	345	15 mgF/L
P07895	Superoxide dismutase [Mn]_ mitochondrial	354	15 mgF/L
B5DF37	TAF6-like RNA polymerase II_ p300/CBP-associated factor (PCAF)-associated factor (Predicted)	62	15 mgF/L
F1M4A0	Tight junction protein 1	21	15 mgF/L
P48500	Triosephosphate isomerase	149	15 mgF/L
F1LNJ2	U5 small nuclear ribonucleoprotein 200 kDa helicase	25	15 mgF/L
D3ZDI9	Ubiquitinyl hydrolase 1	34	15 mgF/L
D4A498	Uncharacterized protein	45	15 mgF/L
M0R8M5	Uncharacterized protein	54	15 mgF/L
A0A0G2JTR5	Uncharacterized protein	65	15 mgF/L
P45953	Very long-chain specific acyl-CoA dehydrogenase_ mitochondrial	41	15 mgF/L
D3ZXU0	Zinc finger with KRAB and SCAN domains 2	35	15 mgF/L
D3ZTP0	10-formyltetrahydrofolate dehydrogenase	41	Control*
G3V965	1-acylglycerol-3-phosphate O-acyltransferase 5	53	Control
Q62878	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 4	116	Control
P97532	3-mercaptopyruvate sulfurtransferase	81	Control
P04644	40S ribosomal protein S17	182	Control
P62909	40S ribosomal protein S3	219	Control
P02401	60S acidic ribosomal protein P2	131	Control
P21531	60S ribosomal protein L3	117	Control
P26769	Adenylate cyclase type 2	39	Control
Q8CGZ2	Afadin- and alpha-actinin-binding protein	70	Control
Q64057	Alpha-amino adipic semialdehyde dehydrogenase	62	Control
F8WFT7	Anion exchange protein	98	Control
Q66H39	ATP-binding cassette sub-family F member 3	68	Control
Q9QY16	ATP-dependent RNA helicase DDX25	80	Control
P23562	Band 3 anion transport protein	98	Control
A0A0G2K728	Cadherin-4	58	Control
Q499T5	Calpain 7	57	Control
Q9ES66	Calpain-10	133	Control
Q6TUG4	Carboxylesterase 1F	73	Control
P16573	Carcinoembryonic antigen-related cell adhesion molecule 1	50	Control
D3Z945	Carnosine synthase 1	131	Control
D4A6H8	Catenin alpha 2	82	Control
F1M6Q2	Ciliary rootlet coiled-coil_ rootletin	26	Control
B5DEL2	Cleavage and polyadenylation-specific factor 1	59	Control

P05179	Cytochrome P450 2C7	274	Control
M0R6M9	Cytochrome P450 3A18	82	Control
Q6P6R2	Dihydrolipoyl dehydrogenase_ mitochondrial	49	Control
Q9EQ76	Dimethylaniline monooxygenase [N-oxide-forming] 3	143	Control
Q9Z330	DNA (cytosine-5)-methyltransferase 1	28	Control
A0A0G2JUY1	E3 ubiquitin-protein ligase	57	Control
Q00911	Early growth response protein 4	200	Control
Q5M884	Enoyl-CoA delta isomerase 2_ mitochondrial	231	Control
A0A0G2K8B7	Eukaryotic initiation factor 4A-II	58	Control
Q6P3V8	Eukaryotic translation initiation factor 4A1	54	Control
Q6AYG3	Exopolyphosphatase PRUNE1	42	Control
Q3T1K5	F-actin-capping protein subunit alpha-2	111	Control
D3ZVN3	F-box protein 10	56	Control
M0R553	Flavin-containing monooxygenase	48	Control
Q6AY07	Fructose-bisphosphate aldolase	38	Control
P05065	Fructose-bisphosphate aldolase A	84	Control
B0BN47	Glutathione S-transferase	211	Control
Q6AXY0	Glutathione S-transferase A6	1107	Control
P14942	Glutathione S-transferase alpha-4	545	Control
O35077	Glycerol-3-phosphate dehydrogenase [NAD(+)]_ cytoplasmic	122	Control
P13255	Glycine N-methyltransferase	464	Control
Q63803	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	35	Control
P63159	High mobility group protein B1	174	Control
D4A7Q7	Hormonally upregulated Neu-associated kinase	55	Control
P97519	Hydroxymethylglutaryl-CoA lyase_ mitochondrial	83	Control
D3ZH46	Inhibitor of Bruton tyrosine kinase	41	Control
Q5PQL7	Integral membrane protein 2C	87	Control
P41562	Isocitrate dehydrogenase [NADP] cytoplasmic	92	Control
P19629	L-lactate dehydrogenase C chain	243	Control
O88989	Malate dehydrogenase_ cytoplasmic	101	Control
F1LRS1	MAP3K12-binding inhibitory protein 1	37	Control
M0RDI5	Mitochondrial calcium uniporter	47	Control
Q9WUJ3	Myomegalin	34	Control
P17209	Myosin light chain 4	113	Control
P04466	Myosin regulatory light chain 2_ skeletal muscle isoform	206	Control
D3ZKE0	N-acetylglucosamine-1-phosphate transferase_ alpha and beta subunits	31	Control
D3ZI75	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2	98	Control
A0A0G2JWA1	Neuroendocrine secretory protein 55	35	Control
Q63369	Nuclear factor NF-kappa-B p105 subunit (Fragment)	71	Control
D3ZEI6	Nuclear receptor coactivator 5	40	Control
P04182	Ornithine aminotransferase_ mitochondrial	214	Control
A2VD13	Pentatricopeptide repeat-containing protein 1_ mitochondrial	70	Control
O35244	Peroxiredoxin-6	67	Control
P31044	Phosphatidylethanolamine-binding protein 1	250	Control
D3ZJG4	Phosphofurin acidic cluster sorting protein 2	53	Control

P16617	Phosphoglycerate kinase 1	72	Control
D3ZZ94	Poly [ADP-ribose] polymerase	57	Control
F1LYX1	Potassium channel tetramerization domain-containing 19	44	Control
F1LTJ8	Pre-mRNA-processing factor 40 homolog B	28	Control
D3ZNI3	Programmed cell death 11	33	Control
D3ZXU4	Protease_serine_42	65	Control
Q9Z213	Protein ALEX	37	Control
P52164	Protein max	70	Control
Q2YDU8	Protein spinster homolog 1	44	Control
F1LP13	Protein tyrosine phosphatase_receptor type_G	50	Control
P12928	Pyruvate kinase PKLR	62	Control
M0R5K3	RCG54339, isoform CRA_c	273	Control
D3ZUE4	RCG61633_ isoform CRA_a	67	Control
D3ZD78	Ribonucleoprotein_PTB-binding 2	61	Control
Q5M9F1	RNA-binding protein 34	115	Control
P05545	Serine protease inhibitor A3K	72	Control
D3ZVH2	Similar to 40S ribosomal protein S3	219	Control
D4A4D5	Similar to 60S acidic ribosomal protein P2	131	Control
D3ZD94	Similar to class-alpha glutathione S-transferase	545	Control
Q63089	Solute carrier family 22 member 1	56	Control
P45380	Sulfate anion transporter 1	107	Control
F1LN91	Tensin 3	41	Control
F1M0Z1	Triple functional domain protein	37	Control
Q68FR8	Tubulin alpha-3 chain	68	Control
Q5XIF6	Tubulin alpha-4A chain	68	Control
Q6AY56	Tubulin alpha-8 chain	60	Control
D3ZF39	UDP-N-acetylglucosamine pyrophosphorylase 1	70	Control
A0A0G2KA73	Uncharacterized protein	174	Control
D3ZXR5	Uncharacterized protein	174	Control
F1MAE5	Uncharacterized protein	58	Control
F1LVK8	Uncharacterized protein	141	Control
A0A0G2K689	Uncharacterized protein	171	Control
A0A0G2K242	Zinc finger protein 516	37	Control

^aIdentification is based on proteins ID from UniProt protein database, reviewed only (<http://www.uniprot.org/>).

^bProteins with expression significantly altered are organized according to the ratio.

*Indicates unique proteins in alphabetical order.

Table 2. Proteins with expression significantly altered in the mitochondria of liver of rats of group treated with 50 mgF/L for 20 days x control group comparison.

^a Accession number	Protein name	PLGS Score	^b Ratio 50 mgF/L: Control
P07872	Peroxisomal acyl-coenzyme A oxidase 1	108	1.16
P15999	ATP synthase subunit alpha_mitochondrial	7255	0.93
P0C0S7	Histone H2A.Z	4377	0.92
P02262	Histone H2A type 1	8057	0.91
A9UMV8	Histone H2A.J	8057	0.91
Q9JM53	Apoptosis-inducing factor 1_mitochondrial	161	0.90

Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_mitochondrial	336	0.90
B2RZD6	NDUFA4_mitochondrial complex-associated	1982	0.90
Q6P9Y4	ADP/ATP translocase 1	377	0.89
Q66HF3	Electron transfer flavoprotein-ubiquinone oxidoreductase_mitochondrial	373	0.89
P67779	Prohibitin	1623	0.89
F1LZW6	Solute carrier family 25 member 13	350	0.89
A0A0G2JSH2	3-hydroxybutyrate dehydrogenase_type 1_isoform CRA_a	421	0.88
P10888	Cytochrome c oxidase subunit 4 isoform 1_mitochondrial	2005	0.88
P29147	D-beta-hydroxybutyrate dehydrogenase_mitochondrial	421	0.88
P16036	Phosphate carrier protein_mitochondrial	708	0.88
Q64428	Trifunctional enzyme subunit alpha_mitochondrial	593	0.87
P05182	Cytochrome P450 2E1	391	0.86
P15650	Long-chain specific acyl-CoA dehydrogenase_mitochondrial	372	0.84
P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	327	0.84
Q9ES38	Bile acyl-CoA synthetase	256	0.83
Q641Y0	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	102	0.82
Q6UPE0	Choline dehydrogenase_mitochondrial	1051	0.81
P05183	Cytochrome P450 3A2	375	0.81
Q68FY0	Cytochrome b-c1 complex subunit 1_mitochondrial	683	0.80
O88752	Epsilon 1 globin	2403	0.80
A0A0G2K7P7	Mitochondrial carrier 2	584	0.80
Q68FU3	Electron transfer flavoprotein subunit beta	559	0.79
A0A0G2JSW3	Globin a4	7854	0.79
P19511	ATP synthase F(0) complex subunit B1_mitochondrial	279	0.78
P10860	Glutamate dehydrogenase 1_mitochondrial	1100	0.78
O89035	Mitochondrial dicarboxylate carrier	406	0.78
Q5XIH7	Prohibitin-2	1071	0.78
P17764	Acetyl-CoA acetyltransferase_mitochondrial	476	0.77
P13803	Electron transfer flavoprotein subunit alpha_mitochondrial	572	0.77
P00481	Ornithine carbamoyltransferase_mitochondrial	760	0.77
P52873	Pyruvate carboxylase_mitochondrial	67	0.77
P11951	Cytochrome c oxidase subunit 6C-2	2479	0.76
Q5BJZ3	Nicotinamide nucleotide transhydrogenase	158	0.76
P26772	10 kDa heat shock protein_mitochondrial	2714	0.76
P18666	Myosin regulatory light chain 12B	623	0.76
Q63716	Peroxisredoxin-1	334	0.76
P97524	Very long-chain acyl-CoA synthetase	154	0.76
P07756	Carbamoyl-phosphate synthase [ammonia]_mitochondrial	3247	0.75
P11240	Cytochrome c oxidase subunit 5A_mitochondrial	1638	0.75
P48721	Stress-70 protein_mitochondrial	80	0.75
A0A0G2K3Z9	Uncharacterized protein	318	0.75
D3ZFAQ	Cytochrome c-1	529	0.74
P13832	Myosin regulatory light chain RLC-A	623	0.74

A0A0G2K6H4	Peroxisomal trans-2-enoyl-CoA reductase	490	0.74
D3ZHA7	Similar to Myosin light chain 1 slow a (Predicted)	203	0.73
G3V734	2_4-dienoyl CoA reductase 1_ mitochondrial_ isoform CRA_a	451	0.73
Q64591	2_4-dienoyl-CoA reductase_ mitochondrial	461	0.73
G3V6T7	Protein disulfide-isomerase A4	154	0.73
P25093	Fumarylacetoacetase	296	0.71
P20788	Cytochrome b-c1 complex subunit Rieske_ mitochondrial	1112	0.70
P55159	Serum paraoxonase/arylesterase 1	468	0.70
P24329	Thiosulfate sulfurtransferase	1235	0.70
Q5M875	17-beta-hydroxysteroid dehydrogenase 13	223	0.70
G3V635	Cytochrome P450 3A18	85	0.70
P23965	Enoyl-CoA delta isomerase 1_ mitochondrial	540	0.70
P30839	Fatty aldehyde dehydrogenase	163	0.70
P04636	Malate dehydrogenase_ mitochondrial	1868	0.70
A0A0G2K2E4	Peptidyl-prolyl cis-trans isomerase	900	0.70
Q68G41	Dodecenoyl-Coenzyme A delta isomerase (3_2 trans- enoyl-Coenzyme A isomerase)	540	0.69
P14604	Enoyl-CoA hydratase_ mitochondrial	244	0.69
P07896	Peroxisomal bifunctional enzyme	1886	0.69
P13437	3-ketoacyl-CoA thiolase_ mitochondrial	679	0.68
P00507	Aspartate aminotransferase_ mitochondrial	547	0.68
P97562	Peroxisomal acyl-coenzyme A oxidase 2	112	0.68
P80254	D-dopachrome decarboxylase	495	0.67
Q00729	Histone H2B type 1-A	2473	0.67
P35435	ATP synthase subunit gamma_ mitochondrial	226	0.66
Q06884	Cytochrome P-450	126	0.66
O88813	Long-chain-fatty-acid--CoA ligase 5	121	0.66
P10111	Peptidyl-prolyl cis-trans isomerase A	1120	0.66
O09171	Betaine--homocysteine S-methyltransferase 1	2501	0.66
P12939	Cytochrome P450 2D10	425	0.66
P04800	Cytochrome P450 3A1	130	0.66
Q510P9	Cytochrome P450_ family 2_ subfamily d_ polypeptide 5	409	0.66
Q6IN39	Hsd17b4 protein	295	0.66
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating]_ mitochondrial	482	0.66
Q62812	Myosin-9	337	0.66
P11884	Aldehyde dehydrogenase_ mitochondrial	324	0.65
P10633	Cytochrome P450 2D1	425	0.65
P97852	Peroxisomal multifunctional enzyme type 2	295	0.65
F1LNW3	Acyl-coenzyme A oxidase	99	0.64
P07824	Arginase-1	6511	0.64
P26453	Basigin	695	0.64
Q64119	Myosin light polypeptide 6	1733	0.64
P06685	Sodium/potassium-transporting ATPase subunit alpha-1	459	0.64
P06687	Sodium/potassium-transporting ATPase subunit alpha-3	245	0.64
P23457	3-alpha-hydroxysteroid dehydrogenase	390	0.63
B2GV99	Myl6 protein	1733	0.63
P06686	Sodium/potassium-transporting ATPase subunit alpha-2	255	0.63

P60711	Actin_ cytoplasmic 1	8996	0.63
G3V7J0	Aldehyde dehydrogenase family 6_ subfamily A1_ isoform CRA_b	482	0.63
P16303	Carboxylesterase 1D	391	0.63
Q9EQS4	Cystathionase (Cystathionine gamma-lyase)	350	0.63
P22791	Hydroxymethylglutaryl-CoA synthase_ mitochondrial	849	0.63
F1LNF0	Myosin heavy chain 14	31	0.63
P63039	60 kDa heat shock protein_ mitochondrial	135	0.62
P18757	Cystathionine gamma-lyase	366	0.62
Q64680	Cytochrome P450 2D4	251	0.62
D3ZB81	Solute carrier family 25 member 31	214	0.62
F1M2N4	Uncharacterized protein	206	0.62
Q06647	ATP synthase subunit O_ mitochondrial	1047	0.61
P09626	Potassium-transporting ATPase alpha chain 1	32	0.61
P31210	3-oxo-5-beta-steroid 4-dehydrogenase	87	0.60
P12938	Cytochrome P450 2D3	372	0.60
Q9JJ40	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	326	0.60
Q03336	Regucalcin	120	0.60
Q498M9	Similar to glyceraldehyde-3-phosphate dehydrogenase	206	0.60
P14740	Dipeptidyl peptidase 4	87	0.59
P20070	NADH-cytochrome b5 reductase 3	312	0.59
P00884	Fructose-bisphosphate aldolase B	2481	0.59
G3V9W6	Aldehyde dehydrogenase	133	0.58
Q66HT1	Fructose-bisphosphate aldolase	2633	0.58
D3ZEN2	Uncharacterized protein	206	0.58
G3V7J5	Carboxylic ester hydrolase	50	0.58
P08011	Microsomal glutathione S-transferase 1	169	0.57
P06761	78 kDa glucose-regulated protein	455	0.56
P04762	Catalase	8094	0.55
P63018	Heat shock cognate 71 kDa protein	104	0.55
P00173	Cytochrome b5	5757	0.55
M0RCB1	Uncharacterized protein	104	0.55
P31399	ATP synthase subunit d_ mitochondrial	850	0.54
P10634	Cytochrome P450 2D26	407	0.54
P19112	Fructose-1_6-bisphosphatase 1	355	0.54
Q64541	Sodium/potassium-transporting ATPase subunit alpha-4	90	0.54
P14659	Heat shock-related 70 kDa protein 2	137	0.54
Q6LED0	Histone H3.1	3178	0.54
P54708	Potassium-transporting ATPase alpha chain 2	68	0.54
P0DMW0	Heat shock 70 kDa protein 1A	198	0.53
D3ZJ08	Histone H3	3178	0.53
G3V8S4	Sodium/potassium-transporting ATPase subunit alpha	68	0.53
P11598	Protein disulfide-isomerase A3	84	0.53
F1LZI1	Similar to heat shock protein 8	76	0.53
A0A0G2JVI3	Uncharacterized protein	93	0.53
M0R8T2	10-formyltetrahydrofolate dehydrogenase	106	0.52
P55063	Heat shock 70 kDa protein 1-like	186	0.52
F1LN91	Tensin 3	41	0.52

A0A0G2K0N8	Uncharacterized protein	2975	0.52
P0DMW1	Heat shock 70 kDa protein 1B	198	0.52
P07871	3-ketoacyl-CoA thiolase B_ peroxisomal	646	0.51
Q66HD0	Endoplasmic	703	0.51
P10719	ATP synthase subunit beta_ mitochondrial	9561	0.51
A0A0G2JSV6	Globin c2	8999	0.51
P04041	Glutathione peroxidase 1	568	0.51
D3ZQT0	Uncharacterized protein	360	0.51
P18163	Long-chain-fatty-acid--CoA ligase 1	2897	0.50
P02692	Fatty acid-binding protein_ liver	2546	0.49
P07632	Superoxide dismutase [Cu-Zn]	2645	0.49
P21775	3-ketoacyl-CoA thiolase A_ peroxisomal	729	0.49
P04797	Glyceraldehyde-3-phosphate dehydrogenase	1509	0.48
P16409	Myosin light chain 3	809	0.48
P04785	Protein disulfide-isomerase	706	0.48
O70127	Bile salt export pump	64	0.47
P62630	Elongation factor 1-alpha 1	1541	0.45
M0R757	Elongation factor 1-alpha	1541	0.45
F1M6C2	Elongation factor 1-alpha 1 pseudogene	1541	0.45
P04903	Glutathione S-transferase alpha-2	1035	0.45
A0A0G2K8S2	Uncharacterized protein	981	0.45
P35565	Calnexin	406	0.44
P09034	Argininosuccinate synthase	247	0.44
P18418	Calreticulin	605	0.44
A0A0G2K9I5	Similar to H3 histone_ family 3B	1666	0.44
Q6AYS8	Estradiol 17-beta-dehydrogenase 11	57	0.43
Q68FT5	S-methylmethionine--homocysteine S-methyltransferase BHMT2	779	0.43
D3ZD94	Similar to class-alpha glutathione S-transferase	545	0.43
P09118	Uricase	18072	0.43
P11883	Aldehyde dehydrogenase_ dimeric NADP-preferring	214	0.42
P00502	Glutathione S-transferase alpha-1	977	0.41
P62632	Elongation factor 1-alpha 2	727	0.41
P04904	Glutathione S-transferase alpha-3	1837	0.41
P14942	Glutathione S-transferase alpha-4	545	0.41
P08683	Cytochrome P450 2C11	228	0.40
P46418	Glutathione S-transferase alpha-5	418	0.39
Q6AXY0	Glutathione S-transferase A6	1107	0.38
D3ZME3	Uncharacterized protein	415	0.38
A0A0G2K7R1	Uncharacterized protein	7927	0.36
P02564	Myosin-7	288	0.34
D3ZLY9	Histone H2B	9048	0.34
Q00715	Histone H2B type 1	9537	0.34
B6RK61	Myosin heavy chain 7B	157	0.34
P02563	Myosin-6	268	0.33
P02600	Myosin light chain 1/3_ skeletal muscle isoform	859	0.32
D3ZRN3	Actin_ beta-like 2	2843	0.31
P63259	Actin_ cytoplasmic 2	8996	0.31

P0C169	Histone H2A type 1-C	8057	0.30
P0CC09	Histone H2A type 2-A	8057	0.30
Q4FZT6	Histone H2A type 3	8057	0.30
P62804	Histone H4	15720	0.30
P12847	Myosin-3	209	0.30
Q29RW1	Myosin-4	390	0.30
P0C170	Histone H2A type 1-E	8057	0.30
Q64598	Histone H2A type 1-F	8057	0.30
Q00728	Histone H2A type 4	8057	0.30
D4AEC0	Histone H2A	4377	0.30
F1LMU0	Myosin heavy chain 1	382	0.29
P84245	Histone H3.3	3274	0.14
P29419	ATP synthase subunit e_mitochondrial	955	50 mgF/L*
D3ZYM6	Calreticulin 4	30	50 mgF/L
O70177	Carboxylic ester hydrolase	35	50 mgF/L
D3ZC16	Dual specificity phosphatase 22 (Predicted)_ isoform CRA_a	223	50 mgF/L
F1LW90	Family with sequence similarity 184_ member A	36	50 mgF/L
P23785	Granulins	111	50 mgF/L
F1LNJ1	Leucine-rich repeat kinase 2	33	50 mgF/L
P42123	L-lactate dehydrogenase B chain	87	50 mgF/L
Q562C4	Methyltransferase-like protein 7B	122	50 mgF/L
Q3KR86	MICOS complex subunit Mic60 (Fragment)	160	50 mgF/L
P97521	Mitochondrial carnitine/acylcarnitine carrier protein	132	50 mgF/L
A0A0G2KAC1	Podocan	41	50 mgF/L
P0CG51	Polyubiquitin-B	282	50 mgF/L
Q63429	Polyubiquitin-C	282	50 mgF/L
A0A1B0GWP8	Protein-glutamate O-methyltransferase	133	50 mgF/L
F1M386	Rap guanine nucleotide exchange factor 2	23	50 mgF/L
A0A0H2UHN7	RCG24013_ isoform CRA_a	35	50 mgF/L
F1M6T3	RCG45278_ isoform CRA_a	36	50 mgF/L
F1M927	Uncharacterized protein	22	50 mgF/L
P16086	Spectrin alpha chain_ non-erythrocytic 1	31	50 mgF/L
P17178	Sterol 26-hydroxylase_ mitochondrial	35	50 mgF/L
D3ZER6	Transportin 2	29	50 mgF/L
F1LU69	Ubiquitin B pseudogene 4	282	50 mgF/L
P62982	Ubiquitin-40S ribosomal protein S27a	282	50 mgF/L
P62986	Ubiquitin-60S ribosomal protein L40	282	50 mgF/L
D3ZTP0	10-formyltetrahydrofolate dehydrogenase	41	Control*
G3V965	1-acylglycerol-3-phosphate O-acyltransferase 5	53	Control
Q63347	26S proteasome regulatory subunit 7	50	Control
Q8CHM7	2-hydroxyacyl-CoA lyase 1	123	Control
P52759	2-iminobutanoate/2-iminopropanoate deaminase	793	Control
Q62878	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4-isomerase type 4	116	Control
P97532	3-mercaptopyruvate sulfurtransferase	81	Control
P04644	40S ribosomal protein S17	182	Control
P62909	40S ribosomal protein S3	219	Control
G3V728	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans)_ isoform CRA_b	192	Control
Q9JLJ3	4-trimethylaminobutyraldehyde dehydrogenase	100	Control
P02401	60S acidic ribosomal protein P2	131	Control
P21531	60S ribosomal protein L3	117	Control

D4A6U1	A disintegrin and metallopeptidase domain 11 (Predicted)_ isoform CRA_a	125	Control
Q7TP88	Ab1-219	397	Control
Q6IMX3	Acetyl-Coenzyme A dehydrogenase_ short chain_ isoform CRA_a	84	Control
P11030	Acyl-CoA-binding protein	1361	Control
F1M9A7	Acyl-coenzyme A oxidase	185	Control
A0A1W2Q6C2	ADAM metallopeptidase domain 11	120	Control
P10760	Adenosylhomocysteinase	63	Control
F1M3G7	A-kinase anchor protein 13	37	Control
Q5I0M4	Aldo-keto reductase family 1_ member C12	89	Control
D3ZPY8	Aldo-keto reductase family 1_ member C13	89	Control
D3ZEL2	Aldo-keto reductase family 1_ member C19	89	Control
P14046	Alpha-1-inhibitor 3	42	Control
Q9QXQ0	Alpha-actinin-4	68	Control
Q64057	Alpha-aminoadipic semialdehyde dehydrogenase	62	Control
P04764	Alpha-enolase	130	Control
G3V8F9	Alpha-methylacyl-CoA racemase	39	Control
Q5U329	Anion exchange protein	98	Control
Q8CGU4	Arf-GAP with GTPase_ ANK repeat and PH domain- containing protein 2	43	Control
P02706	Asialoglycoprotein receptor 1	477	Control
D3ZHR2	ATP-binding cassette subfamily D member 1	38	Control
Q66H39	ATP-binding cassette sub-family F member 3	68	Control
Q9QY16	ATP-dependent RNA helicase DDX25	80	Control
P23562	Band 3 anion transport protein	98	Control
A0A0G2JZD0	Cadherin-4	58	Control
Q499T5	Calpain 7	57	Control
Q9ES66	Calpain-10	133	Control
Q63120	Canalicular multispecific organic anion transporter 1	65	Control
P14141	Carbonic anhydrase 3	236	Control
Q63108	Carboxylesterase 1E	71	Control
Q6TUG4	Carboxylesterase 1F	73	Control
A0A0G2JX75	Carboxylic ester hydrolase	48	Control
P16573	Carcinoembryonic antigen-related cell adhesion molecule 1	50	Control
P32198	Carnitine O-palmitoyltransferase 1_ liver isoform	106	Control
D3Z945	Carnosine synthase 1	131	Control
P22734	Catechol O-methyltransferase	232	Control
Q5U302	Catenin (Cadherin associated protein)_ alpha 1	67	Control
A0A0G2JYF7	Catenin alpha 1	67	Control
D4A6H8	Catenin alpha 2	82	Control
Q9WU82	Catenin beta-1	75	Control
Q7TP12	Cc2-17	71	Control
B5DEL2	Cleavage and polyadenylation-specific factor 1	59	Control
Q0V8T3	Contactin-associated protein like 5-4	38	Control
F1LMS4	Contactin-associated protein-like 5-3	33	Control
B2RYS2	Cytochrome b-c1 complex subunit 7	176	Control
M0R5K3	RCG54339, isoform CRA_c	273	Control
P10715	Cytochrome c_ testis-specific	65	Control
P11711	Cytochrome P450 2A1	122	Control
P15149	Cytochrome P450 2A2	63	Control
P20812	Cytochrome P450 2A3	39	Control
P13107	Cytochrome P450 2B3	187	Control
P11510	Cytochrome P450 2C12_ female-specific	83	Control
P24470	Cytochrome P450 2C23	93	Control
P05179	Cytochrome P450 2C7	274	Control
M0R6M9	Cytochrome P450 3A18	82	Control

P20817	Cytochrome P450 4A14	224	Control
P20816	Cytochrome P450 4A2	92	Control
G3V8J2	Cytochrome P450_ family 8_ subfamily b_ polypeptide 1	106	Control
D4A1V8	DAZ-interacting zinc finger protein 3	52	Control
B2GV15	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	64	Control
Q6P6R2	Dihydrolipoyl dehydrogenase_ mitochondrial	49	Control
P36365	Dimethylaniline monooxygenase [N-oxide-forming] 1	93	Control
Q9EQ76	Dimethylaniline monooxygenase [N-oxide-forming] 3	143	Control
P80067	Dipeptidyl peptidase 1	83	Control
Q9Z330	DNA (cytosine-5)-methyltransferase 1	28	Control
D3ZS06	DNA (cytosine-5)-methyltransferase	26	Control
P11530	Dystrophin	40	Control
A0A0G2JUY1	E3 ubiquitin-protein ligase	57	Control
Q00911	Early growth response protein 4	200	Control
Q641Z6	EH domain-containing protein 1	56	Control
Q8R491	EH domain-containing protein 3	56	Control
Q5RKI1	Eukaryotic initiation factor 4A-II	71	Control
Q6P3V8	Eukaryotic translation initiation factor 4A1	54	Control
Q3T1K5	F-actin-capping protein subunit alpha-2	111	Control
D3ZTJ7	F-box and leucine-rich repeat protein 11 (Predicted)	46	Control
D3ZVN3	F-box protein 10	56	Control
M0R553	Flavin-containing monooxygenase	48	Control
P05065	Fructose-bisphosphate aldolase	84	Control
Q6AY07	Fructose-bisphosphate aldolase A	38	Control
P08033	Gap junction beta-1 protein	276	Control
Q03555	Gephyrin	51	Control
Q62669	Globin a1	53	Control
P24473	Glutathione S-transferase kappa 1	206	Control
P04905	Glutathione S-transferase Mu 1	208	Control
P08010	Glutathione S-transferase Mu 2	177	Control
B0BN47	Glutathione S-transferase	211	Control
P08009	Glutathione S-transferase Yb-3	205	Control
P13255	Glycine N-methyltransferase	464	Control
Q63942	GTP-binding protein Rab-3D	92	Control
Q63803	Guanine nucleotide-binding protein G(s) subunit alpha isoforms XLas	35	Control
F1LST0	HEAT repeat-containing 5B	55	Control
D3ZGW6	High mobility group box 1_ pseudogene 3	174	Control
P63159	High mobility group protein B1	174	Control
D3ZIU9	High mobility group protein B1-like	174	Control
P52925	High mobility group protein B2	174	Control
D3ZCR3	High-mobility group (nonhistone chromosomal) protein 1-like 1	174	Control
A0A0G2K654	Histone cluster 1 H1 family member c	406	Control
D4A3K5	Histone cluster 1 H1 family member d	344	Control
P15865	Histone H1.1	406	Control
P06349	Histone H1.4	736	Control
D4A7Q7	Hormonally upregulated Neu-associated kinase	55	Control
B0BNF9	Hydroxyacid oxidase 1	73	Control
P97519	Hydroxymethylglutaryl-CoA lyase_ mitochondrial	83	Control
D3ZH46	Inhibitor of Bruton tyrosine kinase	41	Control
Q5PQL7	Integral membrane protein 2C	87	Control
G3V8L3	Lamin A_ isoform CRA_b	56	Control
Q64654	Lanosterol 14-alpha demethylase	88	Control
A0A1W2Q6D6	Leucine zipper protein 2	70	Control
F1M0Y2	Leucine-rich repeats and IQ motif-containing 4	43	Control
P19629	L-lactate dehydrogenase C chain	243	Control

Q6P6U3	Lmna protein	56	Control
O88989	Malate dehydrogenase_ cytoplasmic	101	Control
F1LRS1	MAP3K12-binding inhibitory protein 1	37	Control
O88994	Mitochondrial amidoxime reducing component 2	154	Control
M0RDI5	Mitochondrial calcium uniporter	47	Control
M0R6N2	MOSC domain-containing protein 2_ mitochondrial-like	156	Control
Q9WUJ3	Myomegalin	34	Control
Q9JLT0	Myosin-10	72	Control
Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	159	Control
A0A0G2K8W0	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	108	Control
D3ZKE0	N-acetylglucosamine-1-phosphate transferase_ alpha and beta subunits	31	Control
Q561S0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 10_ mitochondrial	402	Control
D3ZI75	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 2	98	Control
Q5BK63	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9_ mitochondrial	247	Control
P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2_ mitochondrial	127	Control
Q641Y2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2_ mitochondrial	41	Control
D4A0T0	NADH:ubiquinone oxidoreductase subunit B10	100	Control
P00388	NADPH--cytochrome P450 reductase	119	Control
A0A0G2JWA1	Neuroendocrine secretory protein 55 OS=Rattus norvegicus GN=Gnas PE=1 SV=1	35	Control
Q63369	Nuclear factor NF-kappa-B p105 subunit (Fragment)	71	Control
D3ZEI6	Nuclear receptor coactivator 5	40	Control
Q05982	Nucleoside diphosphate kinase A	250	Control
P19804	Nucleoside diphosphate kinase B	324	Control
Q497B0	Omega-amidase NIT2	124	Control
P04182	Ornithine aminotransferase_ mitochondrial	214	Control
A2VD13	Pentatricopeptide repeat-containing protein 1_ mitochondrial	70	Control
P24368	Peptidyl-prolyl cis-trans isomerase B	58	Control
Q9Z0V5	Peroxiredoxin-4	118	Control
O35244	Peroxiredoxin-6	67	Control
P31044	Phosphatidylethanolamine-binding protein 1	250	Control
D3ZJG4	Phosphofurin acidic cluster sorting protein 2	53	Control
P16617	Phosphoglycerate kinase 1	72	Control
Q5XIV1	Phosphoglycerate kinase	46	Control
D3ZRP5	Poly [ADP-ribose] polymerase	82	Control
F1LYX1	Potassium channel tetramerization domain-containing 19	44	Control
P48679	Prelamin-A/C	64	Control
F1LTJ8	Pre-mRNA-processing factor 40 homolog B	28	Control
D3ZNI3	Programmed cell death 11	33	Control
D3ZXU4	Protease_ serine_ 42	65	Control
Q5XI79	Protein arginine methyltransferase NDUF7_ mitochondrial	80	Control
Q5I0H9	Protein disulfide-isomerase A5	80	Control
Q63081	Protein disulfide-isomerase A6	251	Control
P52164	Protein max	70	Control
Q2YDU8	Malate dehydrogenase_ cytoplasmic	44	Control
P12928	MAP3K12-binding inhibitory protein 1	62	Control
Q5RKJ9	Mitochondrial amidoxime reducing component 2	171	Control
B0BMW0	RAB14, member RAS oncogene family	92	Control
G3V6H0	MOSC domain-containing protein 2_ mitochondrial-like	171	Control
A0A0G2JTT4	Myomegalin	92	Control
F1LW77	Myosin-10	66	Control

D4A0G7	Malate dehydrogenase_ cytoplasmic	92	Control
D3ZZP2	MAP3K12-binding inhibitory protein 1	86	Control
A0A0G2K201	Mitochondrial amidoxime reducing component 2	86	Control
F1LVC3	RAB6B, member RAS oncogene family	86	Control
P35281	Ras-related protein Rab-10	171	Control
P35284	Ras-related protein Rab-12	92	Control
P35286	Ras-related protein Rab-13	80	Control
P61107	Ras-related protein Rab-14	92	Control
P35289	Ras-related protein Rab-15	147	Control
Q6NYB7	Ras-related protein Rab-1A	435	Control
P10536	Ras-related protein Rab-1B	171	Control
P51156	Ras-related protein Rab-26	92	Control
Q5U316	Ras-related protein Rab-35	184	Control
P63012	Ras-related protein Rab-3A	92	Control
Q63941	Ras-related protein Rab-3B	92	Control
P62824	Ras-related protein Rab-3C	92	Control
Q53B90	Ras-related protein Rab-43	92	Control
P05714	Ras-related protein Rab-4A	92	Control
P51146	Ras-related protein Rab-4B	92	Control
Q9WVB1	Ras-related protein Rab-6A	86	Control
P35280	Ras-related protein Rab-8A	171	Control
P70550	Ras-related protein Rab-8B	171	Control
A0A0H2UHP9	RCG39700_ isoform CRA_d	86	Control
M0R7B4	Histone cluster 1 H1 family member d	406	Control
D3ZAN8	RCG53271	44	Control
D3ZUE4	RCG61633_ isoform CRA_a	67	Control
D3ZD78	Ribonucleoprotein_ PTB-binding 2	61	Control
Q5M9F1	RNA-binding protein 34	115	Control
Q8VIF7	Selenium-binding protein 1	81	Control
P12346	Serotransferrin	132	Control
Q68FP2	Serum paraoxonase/lactonase 3	393	Control
P15651	Short-chain specific acyl-CoA dehydrogenase_ mitochondrial	88	Control
Q63965	Sideroflexin-1	78	Control
D3ZVH2	Similar to 40S ribosomal protein S3	219	Control
D3ZS25	Uncharacterized protein	174	Control
P07340	Sodium/potassium-transporting ATPase subunit beta-1	192	Control
Q9JHZ9	Sodium-coupled neutral amino acid transporter 3	76	Control
P12336	Solute carrier family 2_ facilitated glucose transporter member 2	330	Control
Q63089	Solute carrier family 22 member 1	56	Control
F1LX07	Solute carrier family 25 member 12	62	Control
G3V6S0	Spectrin beta chain	41	Control
B0BMT9	Sqrdl protein	82	Control
D4A5C9	Sterile alpha motif domain-containing 7	78	Control
P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit_ mitochondrial	119	Control
P13086	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha_ mitochondrial	127	Control
F1LPV8	Succinate--CoA ligase [GDP-forming] subunit beta_ mitochondrial	135	Control
P45380	Sulfate anion transporter 1	107	Control
P52847	Sulfotransferase family cytosolic 1B member 1	127	Control
Q66H18	Synaptophysin-like 1	39	Control
A0A0G2K598	Transforming_ acidic coiled-coil-containing protein 2	52	Control
Q07984	Translocon-associated protein subunit delta	101	Control
Q4KLZ6	Triokinase/FMN cyclase	137	Control
F1M0Z1	Triple functional domain protein	37	Control

Q6AY56	Tubulin alpha-8 chain	60	Control
B4F7C2	Tubulin beta chain	61	Control
P85108	Tubulin beta-2A chain	105	Control
Q3KRE8	Tubulin beta-2B chain	105	Control
Q4QRB4	Tubulin beta-3 chain	81	Control
Q6P9T8	Tubulin beta-4B chain	61	Control
P69897	Tubulin beta-5 chain	105	Control
F1LTR3	Tudor domain-containing 12	42	Control
F1MAE5	Uncharacterized protein	58	Control
M0R5J4	Uncharacterized protein	130	Control
D3ZXR5	Uncharacterized protein	174	Control
F1LVK8	Uncharacterized protein	141	Control
Q4KM74	Vesicle-trafficking protein SEC22b	102	Control

^aIdentification is based on proteins ID from UniProt protein database, reviewed only (<http://www.uniprot.org/>).

^bProteins with expression significantly altered are organized according to the ratio.

*Indicates unique proteins in alphabetical order.

Table 3. Proteins with expression significantly altered in the mitochondria of liver of rats of group treated with 15 mgF/L for 60 days x control group comparison.

^a Accession number	Protein name	PLGS Score	^b Ratio 15 mgF/L: Control
D3ZNZ935	Histone H2B	3258	9.78
A0A0G2K7R1	Uncharacterized protein	103	9.68
P26772	10 kDa heat shock protein_ mitochondrial	91	8.25
D3ZD09	Cytochrome c oxidase subunit	102	6.62
P13437	3-ketoacyl-CoA thiolase_ mitochondrial	154	6.42
Q00715	Histone H2B type 1	3258	5.93
Q68G44	Hydroxymethylglutaryl-CoA synthase	145	5.70
P22791	Hydroxymethylglutaryl-CoA synthase_ mitochondrial	145	5.58
P14141	Carbonic anhydrase 3	276	5.16
Q6LED0	Histone H3.1	89	5.05
P84245	Histone H3.3	89	5.00
Q6P9Y4	ADP/ATP translocase 1	514	4.90
P32551	Cytochrome b-c1 complex subunit 2_ mitochondrial	97	4.85
O88752	Epsilon 1 globin	268	4.85
P07756	Carbamoyl-phosphate synthase [ammonia]_ mitochondrial	2600	4.76
P10860	Glutamate dehydrogenase 1_ mitochondrial	1413	4.71
D3ZB81	Solute carrier family 25 member 31	259	4.26
P00406	Cytochrome c oxidase subunit 2	415	4.18
Q68FT5	S-methylmethionine--homocysteine S-methyltransferase BHMT2	229	4.18
P35434	ATP synthase subunit delta_ mitochondrial	401	4.10
Q09073	ADP/ATP translocase 2	411	4.01
P80254	D-dopachrome decarboxylase	578	4.01
A0A0G2JSH2	3-hydroxybutyrate dehydrogenase_ type 1_ isoform CRA_a	134	3.86
P29147	D-beta-hydroxybutyrate dehydrogenase_ mitochondrial	148	3.86
O89035	Mitochondrial dicarboxylate carrier	602	3.78

Q5XIH3	NADH dehydrogenase [ubiquinone] flavoprotein 1_ mitochondrial	245	3.67
P07632	Superoxide dismutase [Cu-Zn]	415	3.63
P45953	Very long-chain specific acyl-CoA dehydrogenase_ mitochondrial	32	3.63
B2GV28	Cyp2b1 protein	21	3.60
P04167	Cytochrome P450 2B2	21	3.60
P08683	Cytochrome P450 2C11	2571	3.60
P15650	Long-chain specific acyl-CoA dehydrogenase_ mitochondrial	186	3.60
P11884	Aldehyde dehydrogenase_ mitochondrial	223	3.53
P00176	Cytochrome P450 2B1	21	3.53
P0C2X9	Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial	109	3.49
P02692	Fatty acid-binding protein_ liver	2143	3.39
P21571	ATP synthase-coupling factor 6_ mitochondrial	208	3.35
O88813	Long-chain-fatty-acid--CoA ligase 5	36	3.35
M0RBS2	Uncharacterized protein	36	3.25
Q9Z2L0	Voltage-dependent anion-selective channel protein 1	714	3.16
P11883	Aldehyde dehydrogenase_ dimeric NADP-preferring	18	3.13
P07824	Arginase-1	1380	3.03
D3ZXZ1	Zinc finger protein 292	30	3.03
Q68FU3	Electron transfer flavoprotein subunit beta	453	2.92
P00481	Ornithine carbamoyltransferase_ mitochondrial	757	2.92
F1LM78	Retinol dehydrogenase 16 (all-trans)	59	2.92
G3V9C0	Histone H2A	749	2.89
P02262	Histone H2A type 1	749	2.89
P0CC09	Histone H2A type 2-A	749	2.89
A9UMV8	Histone H2A.J	749	2.89
P00884	Fructose-bisphosphate aldolase B	336	2.86
P0C169	Histone H2A type 1-C	749	2.86
Q64598	Histone H2A type 1-F	749	2.86
Q4FZT6	Histone H2A type 3	749	2.86
Q00728	Histone H2A type 4	749	2.86
P0C0S7	Histone H2A.Z	749	2.86
P0C170	Histone H2A type 1-E	749	2.83
F7FHR5	RCG60176_ isoform CRA_a	59	2.77
P16303	Carboxylesterase 1D	1220	2.72
B2RZD6	NDUFA4_ mitochondrial complex-associated	457	2.72
P24329	Thiosulfate sulfurtransferase	954	2.72
G3V7J0	Aldehyde dehydrogenase family 6_ subfamily A1_ isoform CRA_b	225	2.69
P20788	Cytochrome b-c1 complex subunit Rieske_ mitochondrial	253	2.69
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating]_ mitochondrial	264	2.66
P04636	Malate dehydrogenase_ mitochondrial	233	2.64
P62804	Histone H4	2372	2.61
P18666	Myosin regulatory light chain 12B	221	2.59
A0A0G2JSV6	Globin c2	1824	2.56
P04797	Glyceraldehyde-3-phosphate dehydrogenase	285	2.56

P13832	Myosin regulatory light chain RLC-A	221	2.56
P14604	Enoyl-CoA hydratase_ mitochondrial	196	2.53
D3ZT90	Glutaryl-CoA dehydrogenase	62	2.53
P25235	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 2	43	2.46
P17764	Acetyl-CoA acetyltransferase_ mitochondrial	271	2.41
A0A0G2JSI0	Flavin-containing monooxygenase	103	2.41
P04762	Catalase	10221	2.39
Q66HT1	Fructose-bisphosphate aldolase	233	2.36
Q498M9	Similar to glyceraldehyde-3-phosphate dehydrogenase	70	2.36
P05182	Cytochrome P450 2E1	274	2.32
A0A0G2K459	Mitochondrial carrier 2	169	2.32
D3ZEN2	Uncharacterized protein	70	2.32
Q9EQ76	Dimethylaniline monooxygenase [N-oxide-forming] 3	103	2.29
P30839	Fatty aldehyde dehydrogenase	481	2.29
P13086	Succinate--CoA ligase [ADP/GDP-forming] subunit alpha_ mitochondrial	93	2.27
O09171	Betaine--homocysteine S-methyltransferase 1	858	2.25
P18163	Long-chain-fatty-acid--CoA ligase 1	3340	2.25
P29419	ATP synthase subunit e_ mitochondrial	526	2.20
P62632	Elongation factor 1-alpha 2	70	2.20
G3V9W6	Aldehyde dehydrogenase	469	2.18
P10888	Cytochrome c oxidase subunit 4 isoform 1_ mitochondrial	847	2.18
P00507	Aspartate aminotransferase_ mitochondrial	382	2.16
P16970	ATP-binding cassette sub-family D member 3	175	2.16
P31399	ATP synthase subunit d_ mitochondrial	672	2.14
Q5XIH7	Prohibitin-2	587	2.14
P11240	Cytochrome c oxidase subunit 5A_ mitochondrial	1105	2.12
A0A0G2K8S2	Uncharacterized protein	196	2.10
FILP50	17-beta-hydroxysteroid dehydrogenase type 6	182	2.05
P21913	Succinate dehydrogenase [ubiquinone] iron-sulfur subunit_ mitochondrial	223	2.05
P19643	Amine oxidase [flavin-containing] B	317	2.03
Q66HD0	Endoplasmic	537	2.03
P04785	Protein disulfide-isomerase	427	2.03
P63039	60 kDa heat shock protein_ mitochondrial	91	2.01
P38659	Protein disulfide-isomerase A4	112	1.99
Q68FY0	Cytochrome b-c1 complex subunit 1_ mitochondrial	1073	1.97
Q6UPE0	Choline dehydrogenase_ mitochondrial	2098	1.95
G3V6I4	Mitochondrial amidoxime reducing component 1	261	1.95
P06761	78 kDa glucose-regulated protein	924	1.93
P10719	ATP synthase subunit beta_ mitochondrial	7482	1.93
Q63965	Sideroflexin-1	131	1.92
P81155	Voltage-dependent anion-selective channel protein 2	514	1.92
P10634	Cytochrome P450 2D26	484	1.90
P22072	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4- isomerase type 2	317	1.88
Q66HF1	NADH-ubiquinone oxidoreductase 75 kDa subunit_ mitochondrial	42	1.88

P11950	Cytochrome c oxidase subunit 6C-1	44	1.86
P12938	Cytochrome P450 2D3	304	1.86
Q63081	Protein disulfide-isomerase A6	308	1.84
P35565	Calnexin	142	1.82
G3V734	2_4-dienoyl CoA reductase 1_ mitochondrial_ isoform CRA_a	67	1.80
Q64591	2_4-dienoyl-CoA reductase_ mitochondrial	67	1.77
P27364	3 beta-hydroxysteroid dehydrogenase type 5	880	1.77
P52873	Pyruvate carboxylase_ mitochondrial	67	1.77
P15999	ATP synthase subunit alpha_ mitochondrial	7317	1.73
P14740	Dipeptidyl peptidase 4	39	1.72
P62630	Elongation factor 1-alpha 1	335	1.72
P21396	Amine oxidase [flavin-containing] A	323	1.70
M0R757	Elongation factor 1-alpha	335	1.70
Q64428	Trifunctional enzyme subunit alpha_ mitochondrial	307	1.70
G3V9Z3	Amine oxidase	323	1.68
Q66HF3	Electron transfer flavoprotein-ubiquinone oxidoreductase_ mitochondrial	187	1.68
F1M6C2	Elongation factor 1-alpha 1 pseudogene	335	1.68
P11951	Cytochrome c oxidase subunit 6C-2	647	1.67
P11598	Protein disulfide-isomerase A3	550	1.67
Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_ mitochondrial	321	1.67
P22071	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4- isomerase type 1	243	1.65
Q62878	3 beta-hydroxysteroid dehydrogenase/Delta 5-->4- isomerase type 4	259	1.65
Q9ES38	Bile acyl-CoA synthetase	443	1.63
P16036	Phosphate carrier protein_ mitochondrial	377	1.62
D3ZFAQ	Cytochrome c-1	502	1.60
Q64680	Cytochrome P450 2D4	90	1.60
P18418	Calreticulin	1125	1.58
D4AA52	Alpha-1-inhibitor III	30	1.55
Q9JM53	Apoptosis-inducing factor 1_ mitochondrial	148	1.55
P18757	Cystathionine gamma-lyase	451	1.55
P00173	Cytochrome b5	5743	1.55
P10633	Cytochrome P450 2D1	334	1.52
Q5XIN6	Mitochondrial proton/calcium exchanger protein	63	1.52
P12939	Cytochrome P450 2D10	333	1.51
Q5I0P9	Cytochrome P450_ family 2_ subfamily d_ polypeptide 5	333	1.51
Q641Y0	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase 48 kDa subunit	167	1.51
P09626	Potassium-transporting ATPase alpha chain 1	59	1.51
Q62812	Myosin-9	66	1.49
Q8K4C0	Dimethylaniline monooxygenase [N-oxide-forming] 5	125	1.46
Q5BJZ3	Nicotinamide nucleotide transhydrogenase	257	1.46
P67779	Prohibitin	977	1.46
F1LNW3	Acyl-coenzyme A oxidase	242	1.45
P20070	NADH-cytochrome b5 reductase 3	554	1.45
D3ZRN3	Actin_ beta-like 2	256	1.43

P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	293	1.43
G3V824	Insulin-like growth factor 2 receptor	26	1.43
Q60587	Trifunctional enzyme subunit beta_ mitochondrial	376	1.43
P97524	Very long-chain acyl-CoA synthetase	192	1.42
Q06647	ATP synthase subunit O_ mitochondrial	938	1.40
Q68G41	Dodecenoyl-Coenzyme A delta isomerase (3_2 trans-enoyl-Coenzyme A isomerase)	337	1.39
P04166	Cytochrome b5 type B	1014	1.36
P23965	Enoyl-CoA delta isomerase 1_ mitochondrial	342	1.36
P06686	Sodium/potassium-transporting ATPase subunit alpha-2	109	1.36
P12346	Serotransferrin	67	1.35
P06685	Sodium/potassium-transporting ATPase subunit alpha-1	181	1.35
B2GV99	Myl6 protein	508	1.32
P06687	Sodium/potassium-transporting ATPase subunit alpha-3	109	1.31
F1LZW6	Solute carrier family 25 member 13	373	1.31
Q00729	Histone H2B type 1-A	3258	1.28
D3ZHA7	Similar to Myosin light chain 1 slow a (Predicted)	144	1.28
Q64119	Myosin light polypeptide 6	508	1.26
D3ZG43	NADH dehydrogenase (Ubiquinone) Fe-S protein 3 (Predicted)_ isoform CRA_c	106	1.25
P10818	Cytochrome c oxidase subunit 6A1_ mitochondrial	713	1.21
P54708	Potassium-transporting ATPase alpha chain 2	109	1.15
Q64541	Sodium/potassium-transporting ATPase subunit alpha-4	117	1.14
G3V8S4	Sodium/potassium-transporting ATPase subunit alpha	109	1.13
P05178	Cytochrome P450 2C6	322	0.66
M0RB90	Cytochrome P450_ family 2_ subfamily C_ polypeptide 6_ variant 1	307	0.66
P11510	Cytochrome P450 2C12_ female-specific	230	0.52
Q6AY30	Saccharopine dehydrogenase-like oxidoreductase	58	0.13
M0R8T2	10-formyltetrahydrofolate dehydrogenase	73	15 mgF/L*
P23457	3-alpha-hydroxysteroid dehydrogenase	307	15 mgF/L
O70351	3-hydroxyacyl-CoA dehydrogenase type-2	1337	15 mgF/L
P97532	3-mercaptopyruvate sulfurtransferase	36	15 mgF/L
Q6TXJ6	40S ribosomal protein S3a	170	15 mgF/L
G3V796	Acetyl-Coenzyme A dehydrogenase_ medium chain	110	15 mgF/L
Q6IMX3	Acetyl-Coenzyme A dehydrogenase_ short chain_ isoform CRA_a	86	15 mgF/L
Q9ER34	Aconitate hydratase_ mitochondrial	64	15 mgF/L
Q6AYT9	Acyl-coenzyme A synthetase ACSM5_ mitochondrial	68	15 mgF/L
P10760	Adenosylhomocysteinase	44	15 mgF/L
P29410	Adenylate kinase 2_ mitochondrial	103	15 mgF/L
Q9WUS0	Adenylate kinase 4_ mitochondrial	177	15 mgF/L
B1WC73	ADP-ribosylation factor-like 6 (Predicted)_ isoform CRA_a	93	15 mgF/L
P38918	Aflatoxin B1 aldehyde reductase member 3	88	15 mgF/L
Q0D2L3	Agmatinase_ mitochondrial	144	15 mgF/L
Q64565	Alanine--glyoxylate aminotransferase 2_ mitochondrial	90	15 mgF/L
D3ZXY4	Aldehyde dehydrogenase 8 family_ member A1	91	15 mgF/L
G3V7I5	Aldehyde dehydrogenase X_ mitochondrial	43	15 mgF/L

D3ZAN3	Alpha glucosidase 2 alpha neutral subunit (Predicted)	107	15 mgF/L
Q9Z1P2	Alpha-actinin-1	58	15 mgF/L
Q64057	Alpha-aminoadipic semialdehyde dehydrogenase	429	15 mgF/L
P04764	Alpha-enolase	86	15 mgF/L
P70473	Alpha-methylacyl-CoA racemase	274	15 mgF/L
A0A0G2JWZ2	Amine oxidase	74	15 mgF/L
F8WFT7	Anion exchange protein	50	15 mgF/L
Q9Z272	ARF GTPase-activating protein GIT1	49	15 mgF/L
Q8CGU4	Arf-GAP with GTPase_ ANK repeat and PH domain-containing protein 2	70	15 mgF/L
P09034	Argininosuccinate synthase	713	15 mgF/L
P13221	Aspartate aminotransferase_ cytoplasmic	66	15 mgF/L
P19511	ATP synthase F(0) complex subunit B1_ mitochondrial	843	15 mgF/L
P35435	ATP synthase subunit gamma_ mitochondrial	345	15 mgF/L
D4AEL4	ATP-binding cassette subfamily B member 5	48	15 mgF/L
A0A0G2K2H0	Axl receptor tyrosine kinase	48	15 mgF/L
Q03248	Beta-ureidopropionase OS=Rattus norvegicus GN=Upb1 PE=1 SV=1	73	15 mgF/L
Q3B8N9	Biphenyl hydrolase-like OS=Rattus norvegicus GN=Bphl PE=1 SV=1	220	15 mgF/L
P55281	Cadherin-17 OS=Rattus norvegicus GN=Cdh17 PE=2 SV=1	52	15 mgF/L
P0DP29	Calmodulin-1	498	15 mgF/L
P0DP30	Calmodulin-2	498	15 mgF/L
P0DP31	Calmodulin-3	527	15 mgF/L
Q63120	Canalicular multispecific organic anion transporter 1	79	15 mgF/L
D4A8A0	Carbamoyl-phosphate synthetase 2_ aspartate transcarbamylase_ and dihydroorotase	45	15 mgF/L
Q6AYS3	Carboxypeptidase	157	15 mgF/L
P00787	Cathepsin B	230	15 mgF/L
Q7TP12	Cc2-17	349	15 mgF/L
O35112	CD166 antigen	71	15 mgF/L
B0K020	CDGSH iron-sulfur domain-containing protein 1	902	15 mgF/L
M0RC85	Centrobin_ centriole duplication and spindle assembly protein	49	15 mgF/L
A0A0G2JXQ2	Chromodomain helicase DNA-binding protein 9	59	15 mgF/L
D3ZUA4	Coiled-coil domain-containing 187	36	15 mgF/L
Q6P6T1	Complement C1s subcomponent	62	15 mgF/L
P01026	Complement C3	87	15 mgF/L
Q64611	Cysteine sulfinic acid decarboxylase	100	15 mgF/L
Q5M9I5	Cytochrome b-c1 complex subunit 6_ mitochondrial	659	15 mgF/L
B2RYS2	Cytochrome b-c1 complex subunit 7	284	15 mgF/L
P12075	Cytochrome c oxidase subunit 5B_ mitochondrial	959	15 mgF/L
P35171	Cytochrome c oxidase subunit 7A2_ mitochondrial	1029	15 mgF/L
P62898	Cytochrome c_ somatic	1354	15 mgF/L
P10715	Cytochrome c_ testis-specific	22	15 mgF/L
P04799	Cytochrome P450 1A2	235	15 mgF/L
P11711	Cytochrome P450 1A1	105	15 mgF/L
P15149	Cytochrome P450 2A2	86	15 mgF/L
P13107	Cytochrome P450 2B3	236	15 mgF/L

P20814	Cytochrome P450 2C13	176	15 mgF/L
P24470	Cytochrome P450 2C23	443	15 mgF/L
P19225	Cytochrome P450 2C70	43	15 mgF/L
P04800	Cytochrome P450 3A1	69	15 mgF/L
Q64581	Cytochrome P450 3A18	77	15 mgF/L
A0A0G2JT98	Cytochrome P450 3A2	69	15 mgF/L
Q06884	Cytochrome P-450	69	15 mgF/L
A0A1B0GWV3	Cytochrome P450_ family 2_ subfamily C_ polypeptide 6_ variant 1 (Fragment)	99	15 mgF/L
D4A253	Cytochrome P450_ family 2_ subfamily c_ polypeptide 7-like	119	15 mgF/L
A0A0G2K283	DEAH (Asp-Glu-Ala-His) box polypeptide 8	59	15 mgF/L
Q6TXG4	DEAH-box helicase 8	54	15 mgF/L
Q6I7R1	Dehydrogenase/reductase (SDR family) member 7	517	15 mgF/L
M0R4N4	Dehydrogenase/reductase (SDR family) member 7-like 1	536	15 mgF/L
B2GV15	Dihydrolipoamide acetyltransferase component of pyruvate dehydrogenase complex	75	15 mgF/L
Q6P6R2	Dihydrolipoyl dehydrogenase_ mitochondrial	85	15 mgF/L
Q01205	Dihydrolipoyllysine-residue succinyltransferase component of 2-oxoglutarate dehydrogenase complex_ mitochondrial	130	15 mgF/L
P11348	Dihydropteridine reductase	221	15 mgF/L
Q63150	Dihydropyrimidinase	89	15 mgF/L
P36365	Dimethylaniline monooxygenase [N-oxide-forming] 1	59	15 mgF/L
Q5RKL4	Dimethylglycine dehydrogenase	47	15 mgF/L
Q63342	Dimethylglycine dehydrogenase_ mitochondrial	68	15 mgF/L
P80067	Dipeptidyl peptidase 1	144	15 mgF/L
Q9EPB1	Dipeptidyl peptidase 2	139	15 mgF/L
A0A0G2JY22	Disco-interacting protein 2 homolog B	34	15 mgF/L
D3ZZB0	Disco-interacting protein 2 homolog C	29	15 mgF/L
Q63202	Disintegrin and metalloproteinase domain-containing protein 2	36	15 mgF/L
G3V678	DNA-directed RNA polymerases I_ II_ and III subunit RPABC3-like	68	15 mgF/L
P13803	Electron transfer flavoprotein subunit alpha_ mitochondrial	1106	15 mgF/L
P05197	Elongation factor 2	27	15 mgF/L
Q5XIC0	Enoyl-CoA delta isomerase 2_ mitochondrial	300	15 mgF/L
Q5RKI1	Eukaryotic initiation factor 4A-II	54	15 mgF/L
Q6IV68	Fanconi anemia group D2 protein homolog	39	15 mgF/L
F1M0R6	Fanconi anemia_ complementation group F	138	15 mgF/L
D3ZBM3	Ferrochelataase	51	15 mgF/L
Q5XI81	Fragile X mental retardation syndrome-related protein 1	61	15 mgF/L
P19112	Fructose-1_6-bisphosphatase 1	386	15 mgF/L
D4ABT6	G protein-coupled receptor 153	64	15 mgF/L
P08033	Gap junction beta-1 protein	370	15 mgF/L
P04041	Glutathione peroxidase 1	747	15 mgF/L
Q6AXY0	Glutathione S-transferase A6	357	15 mgF/L
P00502	Glutathione S-transferase alpha-1	1723	15 mgF/L
P04903	Glutathione S-transferase alpha-2	1807	15 mgF/L
P04904	Glutathione S-transferase alpha-3	1381	15 mgF/L

P14942	Glutathione S-transferase alpha-4	228	15 mgF/L
P46418	Glutathione S-transferase alpha-5	240	15 mgF/L
P24473	Glutathione S-transferase kappa 1	264	15 mgF/L
P04905	Glutathione S-transferase Mu 1	566	15 mgF/L
P08010	Glutathione S-transferase Mu 2	49	15 mgF/L
Q9Z1B2	Glutathione S-transferase Mu 5	41	15 mgF/L
P08009	Glutathione S-transferase Yb-3	351	15 mgF/L
D3ZVQ8	Glutathione S-transferase_ mu 6-like	41	15 mgF/L
O35077	Glycerol-3-phosphate dehydrogenase [NAD(+)]_ cytoplasmic	160	15 mgF/L
Q63942	GTP-binding protein Rab-3D	198	15 mgF/L
P06866	Haptoglobin	108	15 mgF/L
F1LSK5	HEAT repeat-containing 5A	47	15 mgF/L
P0DMW0	Heat shock 70 kDa protein 1A	72	15 mgF/L
P0DMW1	Heat shock 70 kDa protein 1B	72	15 mgF/L
P55063	Heat shock 70 kDa protein 1-like	72	15 mgF/L
P63018	Heat shock cognate 71 kDa protein	311	15 mgF/L
P14659	Heat shock-related 70 kDa protein 2	215	15 mgF/L
D4A974	HIC ZBTB transcriptional repressor 1	51	15 mgF/L
M0R6X5	Histone H2A	65	15 mgF/L
A0A0G2K5X7	Histone H3	35	15 mgF/L
B0BNF9	Hydroxyacid oxidase 1	537	15 mgF/L
Q9WVK7	Hydroxyacyl-coenzyme A dehydrogenase_ mitochondrial	1813	15 mgF/L
P17425	Hydroxymethylglutaryl-CoA synthase_ cytoplasmic	86	15 mgF/L
Q2V057	Hydroxyproline dehydrogenase	196	15 mgF/L
A0A0G2K7D6	Hypermethylated in cancer 1 protein-like	51	15 mgF/L
P56574	Isocitrate dehydrogenase [NADP]_ mitochondrial	90	15 mgF/L
P12007	Isovaleryl-CoA dehydrogenase_ mitochondrial	176	15 mgF/L
O88867	Kynurenine 3-monooxygenase	74	15 mgF/L
Q64602	Kynurenine/alpha-aminoadipate aminotransferase_ mitochondrial	228	15 mgF/L
Q58FK9	Kynurenine--oxoglutarate transaminase 3	224	15 mgF/L
D3ZFJ6	Lactamase_ beta	246	15 mgF/L
P10867	L-gulonolactone oxidase	127	15 mgF/L
P04642	L-lactate dehydrogenase A chain	465	15 mgF/L
P42123	L-lactate dehydrogenase B chain	125	15 mgF/L
P19629	L-lactate dehydrogenase C chain	52	15 mgF/L
O88989	Malate dehydrogenase_ cytoplasmic	203	15 mgF/L
P57113	Maleylacetoacetate isomerase	188	15 mgF/L
P08503	Medium-chain specific acyl-CoA dehydrogenase_ mitochondrial	119	15 mgF/L
O08590	Membrane primary amine oxidase	74	15 mgF/L
Q3KR86	MICOS complex subunit MIC60	86	15 mgF/L
P08011	Microsomal glutathione S-transferase 1	1177	15 mgF/L
A0A0G2K9L9	Midasin AAA ATPase 1	64	15 mgF/L
O88994	Mitochondrial amidoxime reducing component 2	82	15 mgF/L
P97521	Mitochondrial carnitine/acylcarnitine carrier protein	170	15 mgF/L
Q5FVG4	Mitochondrial glutamate carrier 1-like	209	15 mgF/L
Q505J6	Mitochondrial glutamate carrier 2	120	15 mgF/L

Q63538	Mitogen-activated protein kinase 12	67	15 mgF/L
M0R6N2	MOSC domain-containing protein 2_ mitochondrial-like	69	15 mgF/L
B0BMS8	Myl9 protein	41	15 mgF/L
Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	75	15 mgF/L
Q9JJ40	Na(+)/H(+) exchange regulatory cofactor NHE-RF3	210	15 mgF/L
Q5RJN0	NADH dehydrogenase (Ubiquinone) Fe-S protein 7	154	15 mgF/L
F1LXA0	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 12	259	15 mgF/L
Q5BK63	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9_ mitochondrial	425	15 mgF/L
Q5PQZ9	NADH dehydrogenase [ubiquinone] 1 subunit C2	91	15 mgF/L
P19234	NADH dehydrogenase [ubiquinone] flavoprotein 2_ mitochondrial	327	15 mgF/L
Q641Y2	NADH dehydrogenase [ubiquinone] iron-sulfur protein 2_ mitochondrial	222	15 mgF/L
D4A0T0	NADH:ubiquinone oxidoreductase subunit B10	141	15 mgF/L
P00388	NADPH--cytochrome P450 reductase	269	15 mgF/L
P11915	Non-specific lipid-transfer protein	74	15 mgF/L
O88984	Nuclear RNA export factor 1	34	15 mgF/L
Q05982	Nucleoside diphosphate kinase A	353	15 mgF/L
P19804	Nucleoside diphosphate kinase B	353	15 mgF/L
G3V816	Nucleoside diphosphate kinase	174	15 mgF/L
Q497B0	Omega-amidase NIT2	243	15 mgF/L
P04182	Ornithine aminotransferase_ mitochondrial	194	15 mgF/L
G3V710	Peroxiredoxin 3	185	15 mgF/L
Q63716	Peroxiredoxin-1	390	15 mgF/L
Q9Z0V5	Peroxiredoxin-4	171	15 mgF/L
Q9R063	Peroxiredoxin-5_ mitochondrial	376	15 mgF/L
Q6AYW2	Phenylalanine hydroxylase	211	15 mgF/L
P04176	Phenylalanine-4-hydroxylase	215	15 mgF/L
F1LY61	Phospholipase A2_ group IVC	55	15 mgF/L
D3ZUF9	Pitrilysin metallopeptidase 1 (Predicted)	40	15 mgF/L
D4A651	PMS1 homolog 1_ mismatch repair system component	40	15 mgF/L
B1H246	PMS1 homolog 2_ mismatch repair system component	56	15 mgF/L
F1LQ55	Podocan	74	15 mgF/L
F1LZ05	Poly [ADP-ribose] polymerase	58	15 mgF/L
Q4KLP0	Probable 2-oxoglutarate dehydrogenase E1 component DHKTD1_ mitochondrial	154	15 mgF/L
P00786	Pro-cathepsin H	167	15 mgF/L
M0RAK0	Proline and serine-rich 2	403	15 mgF/L
F1MAR6	Proline dehydrogenase	153	15 mgF/L
Q9JHW0	Proteasome subunit beta type-7	60	15 mgF/L
Q64617	Protein kinase C eta type	40	15 mgF/L
F1LS01	Protocadherin 9	69	15 mgF/L
Q5RKJ9	RAB10_ member RAS oncogene family	325	15 mgF/L
B0BMW0	RAB14_ member RAS oncogene family	198	15 mgF/L
E9PU16	RAB1A_ member RAS oncogene family	325	15 mgF/L
G3V6H0	RAB1B_ member RAS oncogene family-like	325	15 mgF/L
A0A0G2JTT4	RAB30_ member RAS oncogene family	198	15 mgF/L
F1LW77	RAB33B_ member RAS oncogene family	226	15 mgF/L

D4A0G7	RAB37_ member RAS oncogene family	198	15 mgF/L
D3ZZP2	RAB39_ member RAS oncogene family (Predicted)	258	15 mgF/L
A0A0G2K201	RAB42_ member RAS oncogene family	258	15 mgF/L
F1LVC3	RAB6B_ member RAS oncogene family	258	15 mgF/L
P35281	Ras-related protein Rab-10	325	15 mgF/L
P35284	Ras-related protein Rab-12	198	15 mgF/L
D4A376	Ras-related protein Rab-12	198	15 mgF/L
P35286	Ras-related protein Rab-13	127	15 mgF/L
P61107	Ras-related protein Rab-14	198	15 mgF/L
P35289	Ras-related protein Rab-15	297	15 mgF/L
Q6NYB7	Ras-related protein Rab-1A	325	15 mgF/L
P10536	Ras-related protein Rab-1B	325	15 mgF/L
P51156	Ras-related protein Rab-26	198	15 mgF/L
Q5U316	Ras-related protein Rab-35	328	15 mgF/L
P63012	Ras-related protein Rab-3A	198	15 mgF/L
Q63941	Ras-related protein Rab-3B	198	15 mgF/L
P62824	Ras-related protein Rab-3C	198	15 mgF/L
Q53B90	Ras-related protein Rab-43	198	15 mgF/L
P05714	Ras-related protein Rab-4A	198	15 mgF/L
P51146	Ras-related protein Rab-4B	198	15 mgF/L
Q9WVB1	Ras-related protein Rab-6A	258	15 mgF/L
P35280	Ras-related protein Rab-8A	325	15 mgF/L
P70550	Ras-related protein Rab-8B	325	15 mgF/L
A0A0H2UHN7	RCG24013_ isoform CRA_a	215	15 mgF/L
B0BNM4	RCG33221_ isoform CRA_c	53	15 mgF/L
A0A0H2UHP9	RCG39700_ isoform CRA_d	258	15 mgF/L
F1LRY5	RCG45398	36	15 mgF/L
Q03336	Regucalcin	106	15 mgF/L
Q5XI73	Rho GDP-dissociation inhibitor 1	150	15 mgF/L
D3ZSB7	Ribosomal protein S6 kinase	33	15 mgF/L
F1LZ34	S-adenosylmethionine synthase	130	15 mgF/L
Q64380	Sarcosine dehydrogenase_ mitochondrial	48	15 mgF/L
P70584	Short/branched chain specific acyl-CoA dehydrogenase_ mitochondrial	53	15 mgF/L
P15651	Short-chain specific acyl-CoA dehydrogenase_ mitochondrial	86	15 mgF/L
D4ACC5	Similar to 106 kDa O-GlcNAc transferase-interacting protein (Predicted)_ isoform CRA_a	58	15 mgF/L
D3ZD94	Similar to class-alpha glutathione S-transferase	228	15 mgF/L
D4A5L9	Similar to Cytochrome c_ somatic	1354	15 mgF/L
F1LZI1	Similar to heat shock protein 8	100	15 mgF/L
M0R947	Similar to tripartite motif-containing 62	58	15 mgF/L
Q63089	Solute carrier family 22 member 1	73	15 mgF/L
A0A0G2K309	Solute carrier family 25 (Mitochondrial carrier; ornithine transporter) member 15	333	15 mgF/L
F1LX07	Solute carrier family 25 member 12	347	15 mgF/L
D4A678	Spectrin_ alpha_ erythrocytic 1	36	15 mgF/L
Q9JKL7	Splicing regulatory glutamine/lysine-rich protein 1	60	15 mgF/L
B0BMT9	Sqrdl protein	386	15 mgF/L

P48721	Stress-70 protein_mitochondrial	200	15 mgF/L
Q641Z9	Succinate dehydrogenase complex subunit C	149	15 mgF/L
F1LPV8	Succinate--CoA ligase [GDP-forming] subunit beta_mitochondrial	79	15 mgF/L
P45380	Sulfate anion transporter 1	68	15 mgF/L
Q07116	Sulfite oxidase_mitochondrial	305	15 mgF/L
P07895	Superoxide dismutase [Mn]_mitochondrial	341	15 mgF/L
Q9Z0V6	Thioredoxin-dependent peroxide reductase_mitochondrial	185	15 mgF/L
P17246	Transforming growth factor beta-1	95	15 mgF/L
P32089	Tricarboxylate transport protein_mitochondrial	249	15 mgF/L
B4F7C2	Tubulin beta chain	130	15 mgF/L
P85108	Tubulin beta-2A chain	50	15 mgF/L
Q3KRE8	Tubulin beta-2B chain	50	15 mgF/L
Q6P9T8	Tubulin beta-4B chain	141	15 mgF/L
P69897	Tubulin beta-5 chain	50	15 mgF/L
Q9QWG5	Type II inositol 3_4-bisphosphate 4-phosphatase	43	15 mgF/L
Q07014	Tyrosine-protein kinase Lyn	30	15 mgF/L
Q66H62	Ubiquitin carboxyl-terminal hydrolase CYLD	175	15 mgF/L
Q66H33	Uncharacterized protein C3orf38 homolog	38	15 mgF/L
M0R5J4	Uncharacterized protein	86	15 mgF/L
M0RCB1	Uncharacterized protein	307	15 mgF/L
P98166	Very low-density lipoprotein receptor	61	15 mgF/L
Q9R1Z0	Voltage-dependent anion-selective channel protein 3	505	15 mgF/L
E9PSN4	Zinc finger CCCH type-containing 13	69	15 mgF/L
D4A069	Zinc finger MYM-type-containing 4	51	15 mgF/L
P35738	2-oxoisovalerate dehydrogenase subunit beta_mitochondrial	100	Control*
P25409	Alanine aminotransferase 1	73	Control
G3V7V6	All-trans-13_14-dihydroretinol saturase_isoform CRA_b	81	Control
Q8VHE9	All-trans-retinol 13_14-reductase	81	Control
G3V8Y7	AP-4 complex accessory subunit Tepsin	40	Control
D3ZHR2	ATP-binding cassette subfamily D member 1	60	Control
D4A8B1	Bardet-Biedl syndrome 4	52	Control
D4A3M7	BSD domain-containing 1	82	Control
D4AC89	BTB domain-containing 11	34	Control
A0A0G2K9E3	BTB domain-containing 7	37	Control
Q5DWV2	Cadherin-7	94	Control
D3ZQR3	cAMP-regulated phosphoprotein 21	47	Control
Q9WU82	Catenin beta-1	66	Control
D3ZPR0	Chromosome segregation 1-like	121	Control
Q5SGD7	Connector enhancer of kinase suppressor of ras 3	29	Control
Q6P4Z9	COP9 signalosome complex subunit 8	95	Control
A0A0G2K173	Cytochrome P450 1B1	50	Control
P20817	Cytochrome P450 4A14	152	Control
P20816	Cytochrome P450 4A2	130	Control
D3ZD73	DEAD-box helicase 6	33	Control
D4A2Z8	DEAH (Asp-Glu-Ala-His) box polypeptide 36 (Predicted)_isoform CRA_a	38	Control

Q64346	Dual specificity protein phosphatase 6	136	Control
D3ZPD6	Fez family zinc finger 1	54	Control
F1LVZ9	HECT domain E3 ubiquitin protein ligase 3	70	Control
Q6IE24	Inactive ubiquitin carboxyl-terminal hydrolase 54	23	Control
Q9JK71	Membrane-associated guanylate kinase_ WW and PDZ domain-containing protein 3	37	Control
E9PTQ5	Methyl-CpG binding domain protein 5	36	Control
Q0D2L2	Mitochondrial ribosomal protein S22	79	Control
D3ZHL6	Mitogen-activated protein kinase kinase kinase	43	Control
E9PT87	Myosin light chain kinase 3	33	Control
D3Z8Q6	Poly [ADP-ribose] polymerase	31	Control
Q5FVJ0	Protein RUFY3	39	Control
G3V9S3	Protein tyrosine phosphatase_ non-receptor type 13	53	Control
Q5M9F1	RNA-binding protein 34	91	Control
P11507	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	34	Control
Q5U311	Schlafen 8	37	Control
G3V9Q6	Similar to putative TRAF and TNF receptor associated protein_ isoform CRA_c	79	Control
A0A0G2JZ18	TEN1 CST complex subunit	185	Control
D4A709	Tubulin_ gamma complex-associated protein 6	32	Control
Q3T1H5	Tyrosyl-DNA phosphodiesterase 2	79	Control
D3ZLQ8	Ubiquitinyl hydrolase 1	56	Control
D3ZSL9	Very long-chain acyl-CoA synthetase	29	Control

^aIdentification is based on proteins ID from UniProt protein database, reviewed only (<http://www.uniprot.org/>).

^bProteins with expression significantly altered are organized according to the ratio.

*Indicates unique proteins in alphabetical order.

Table 4. Proteins with expression significantly altered in the mitochondria of liver of rats of group treated with 50 mgF/L for 60 days x control group comparison.

^a Accession number	Protein name	PLGS Score	^b Ratio 50 mgF/L: Control
P26772	10 kDa heat shock protein_ mitochondrial	91	2.12
D3ZHL6	Mitogen-activated protein kinase kinase kinase	43	1.86
Q68G44	Hydroxymethylglutaryl-CoA synthase	145	1.84
P80254	D-dopachrome decarboxylase	578	1.72
P0C2X9	Delta-1-pyrroline-5-carboxylate dehydrogenase_ mitochondrial	109	1.67
P04797	Glyceraldehyde-3-phosphate dehydrogenase	285	1.67
P26453	Basigin	372	1.65
P00884	Fructose-bisphosphate aldolase B	336	1.60
P22791	Hydroxymethylglutaryl-CoA synthase_ mitochondrial	145	1.58
Q66HT1	Fructose-bisphosphate aldolase	233	1.54
P09626	Potassium-transporting ATPase alpha chain 1	59	1.54
O09171	Betaine--homocysteine S-methyltransferase 1	858	1.49
A0A0G2K7R1	Uncharacterized protein	103	1.49
D3ZD09	Cytochrome c oxidase subunit	102	1.46
D3ZSB0	Cytochrome c oxidase subunit 6B1	102	1.46
Q5FVR5	Acyl-coenzyme A amino acid N-acyltransferase 2	65	1.43

A0A0G2JYY8	Uncharacterized protein OS=Rattus norvegicus PE=1 SV=1	125	1.43
P02692	Fatty acid-binding protein_liver	2143	1.40
B2RZD6	NDUFA4_mitochondrial complex-associated	457	1.40
P07824	Arginase-1	1380	1.38
P18666	Myosin regulatory light chain 12B	221	1.38
D3ZME3	Uncharacterized protein	125	1.38
P04636	Malate dehydrogenase_mitochondrial	233	1.36
F7FHR5	RCG60176_isoform CRA_a	59	1.36
Q64541	Sodium/potassium-transporting ATPase subunit alpha-4	117	1.36
P17764	Acetyl-CoA acetyltransferase_mitochondrial	271	1.35
P10860	Glutamate dehydrogenase 1_mitochondrial	1413	1.35
Q62812	Myosin-9	66	1.35
G3V6P7	Myosin_heavy polypeptide 9_non-muscle	72	1.32
A0A0G2K8S2	Uncharacterized protein	196	1.32
P14740	Dipeptidyl peptidase 4	39	1.30
Q64119	Myosin light polypeptide 6	508	1.30
P54708	Potassium-transporting ATPase alpha chain 2	109	1.28
G3V8S4	Sodium/potassium-transporting ATPase subunit alpha	109	1.28
P00507	Aspartate aminotransferase_mitochondrial	382	1.27
P15650	Long-chain specific acyl-CoA dehydrogenase_mitochondrial	186	1.27
Q6P9Y4	ADP/ATP translocase 1	514	1.26
P12938	Cytochrome P450 2D3	304	1.23
Q02253	Methylmalonate-semialdehyde dehydrogenase [acylating]_mitochondrial	264	1.23
P04166	Cytochrome b5 type B	1014	1.21
P07687	Epoxide hydrolase 1	1257	1.21
G3V7J0	Aldehyde dehydrogenase family 6_subfamily A1_ isoform CRA_b	225	1.20
P00406	Cytochrome c oxidase subunit 2	415	1.20
P11884	Aldehyde dehydrogenase_mitochondrial	223	1.19
P06685	Sodium/potassium-transporting ATPase subunit alpha-1	181	1.17
P30839	Fatty aldehyde dehydrogenase	481	1.16
P10633	Cytochrome P450 2D1	334	1.15
Q5I0P9	Cytochrome P450_family 2_subfamily d_polypeptide 5	333	1.15
P18757	Cystathionine gamma-lyase	451	1.14
P12939	Cytochrome P450 2D10	333	1.14
P13437	3-ketoacyl-CoA thiolase_mitochondrial	154	1.13
G3V9W6	Aldehyde dehydrogenase	469	1.12
P07153	Dolichyl-diphosphooligosaccharide--protein glycosyltransferase subunit 1	293	1.12
P20070	NADH-cytochrome b5 reductase 3	554	1.12
P27364	3 beta-hydroxysteroid dehydrogenase type 5	880	1.11
P60711	Actin_cytoplasmic 1	3508	1.06
P63259	Actin_cytoplasmic 2	3508	1.06
F1LP05	ATP synthase subunit alpha	7316	0.96
P15999	ATP synthase subunit alpha_mitochondrial	7317	0.96
P21396	Amine oxidase [flavin-containing] A	323	0.91

A0A0G2K099	Uncharacterized protein	2396	0.90
P67779	Prohibitin	977	0.89
Q60587	Trifunctional enzyme subunit beta_ mitochondrial	376	0.89
F1LZW6	Solute carrier family 25 member 13	373	0.88
Q9ES38	Bile acyl-CoA synthetase	443	0.87
P18418	Calreticulin	1125	0.87
Q5BJZ3	Nicotinamide nucleotide transhydrogenase	257	0.84
Q920L2	Succinate dehydrogenase [ubiquinone] flavoprotein subunit_ mitochondrial	321	0.84
Q64428	Trifunctional enzyme subunit alpha_ mitochondrial	307	0.84
A0A0G2K7P7	Mitochondrial carrier 2	269	0.83
P63039	60 kDa heat shock protein_ mitochondrial	91	0.80
Q6UPE0	Choline dehydrogenase_ mitochondrial	2098	0.80
P11598	Protein disulfide-isomerase A3	550	0.79
Q5XIH7	Prohibitin-2	587	0.79
P06761	78 kDa glucose-regulated protein	924	0.77
P10719	ATP synthase subunit beta_ mitochondrial	7482	0.77
D3ZFAQ8	Cytochrome c-1	502	0.77
P16970	ATP-binding cassette sub-family D member 3	175	0.76
P11240	Cytochrome c oxidase subunit 5A_ mitochondrial	1105	0.76
P35565	Calnexin	142	0.72
P04785	Protein disulfide-isomerase	427	0.72
P18163	Long-chain-fatty-acid--CoA ligase 1	3340	0.65
P07632	Superoxide dismutase [Cu-Zn]	415	0.64
G3V6D3	ATP synthase subunit beta	7482	0.63
D3ZRN3	Actin_ beta-like 2	256	0.62
P24329	Thiosulfate sulfurtransferase	954	0.60
P07756	Carbamoyl-phosphate synthase [ammonia]_ mitochondrial	2600	0.57
P00173	Cytochrome b5	5743	0.57
Q00729	Histone H2B type 1-A	3258	0.49
P08683	Cytochrome P450 2C11	2571	0.46
O70351	3-hydroxyacyl-CoA dehydrogenase type-2	523	50 mgF/L*
Q6TXJ6	40S ribosomal protein S3a	96	50 mgF/L
P11030	Acyl-CoA-binding protein	393	50 mgF/L
A0A0G2K2H6	Acyl-coenzyme A amino acid N-acyltransferase 1	91	50 mgF/L
P09034	Argininosuccinate synthase	73	50 mgF/L
P02706	Asialoglycoprotein receptor 1	129	50 mgF/L
P35435	ATP synthase subunit gamma_ mitochondrial	95	50 mgF/L
Q9Z1Y3	Cadherin-2	45	50 mgF/L
P0DP29	Calmodulin-1	251	50 mgF/L
P0DP30	Calmodulin-2	251	50 mgF/L
P0DP31	Calmodulin-3	263	50 mgF/L
A0A0G2KB83	Carboxylic ester hydrolase	24	50 mgF/L
Q5M9I5	Cytochrome b-c1 complex subunit 6_ mitochondrial	730	50 mgF/L

P12075	Cytochrome c oxidase subunit 5B_mitochondrial	216	50 mgF/L
P35171	Cytochrome c oxidase subunit 7A2_mitochondrial	595	50 mgF/L
P62898	Cytochrome c_somatic	190	50 mgF/L
M0R4N4	Dehydrogenase/reductase (SDR family) member 7-like 1	180	50 mgF/L
D3ZQ89	E1A binding protein p400_isoform CRA_a	28	50 mgF/L
P13803	Electron transfer flavoprotein subunit alpha_mitochondrial	486	50 mgF/L
P19112	Fructose-1_6-bisphosphatase 1	128	50 mgF/L
P04642	L-lactate dehydrogenase A chain	168	50 mgF/L
P97521	Mitochondrial carnitine/acylcarnitine carrier protein	302	50 mgF/L
Q5XIS8	Mitochondrial dynamics protein MID51	44	50 mgF/L
E9PSK7	Mitogen-activated protein kinase 8-interacting protein 3	60	50 mgF/L
B0BMS8	Myl9 protein	23	50 mgF/L
P12847	Myosin-3	22	50 mgF/L
Q9JJ19	Na(+)/H(+) exchange regulatory cofactor NHE-RF1	71	50 mgF/L
Q5BK63	NADH dehydrogenase [ubiquinone] 1 alpha subcomplex subunit 9_mitochondrial	197	50 mgF/L
Q63372	Neurexin-1	64	50 mgF/L
Q497B0	Omega-amidase NIT2	92	50 mgF/L
P04182	Ornithine aminotransferase_mitochondrial	154	50 mgF/L
A0A0G2K2E4	Peptidyl-prolyl cis-trans isomerase	178	50 mgF/L
A0A0G2K1P0	Peptidyl-prolyl cis-trans isomerase	236	50 mgF/L
P10111	Peptidyl-prolyl cis-trans isomerase A	236	50 mgF/L
D3ZFC5	Phospholipid-transporting ATPase	25	50 mgF/L
Q9JKB0	Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 1	30	50 mgF/L
D4A7P5	Protocadherin beta 20	25	50 mgF/L
Q03336	Regucalcin	324	50 mgF/L
D4A5L9	Similar to Cytochrome c_somatic	190	50 mgF/L
Q3T1H9	Similar to Rnf37-pending protein	28	50 mgF/L
P12336	Solute carrier family 2_facilitated glucose transporter member 2	73	50 mgF/L
D3Z9Z3	SPG11_spatacsin vesicle-trafficking-associated	11	50 mgF/L
Q66H62	Ubiquitin carboxyl-terminal hydrolase CYLD	98	50 mgF/L
Q9Z270	Vesicle-associated membrane protein-associated protein A	130	50 mgF/L
D3ZI38	Zinc finger_SWIM domain containing 5 (Predicted)	23	50 mgF/L
Q5M875	17-beta-hydroxysteroid dehydrogenase 13	147	Control*
O54753	17-beta-hydroxysteroid dehydrogenase type 6	182	Control
P35738	2-oxoisovalerate dehydrogenase subunit beta_mitochondrial	100	Control
P32755	4-hydroxyphenylpyruvate dioxygenase	75	Control
G3V728	4-nitrophenylphosphatase domain and non-neuronal SNAP25-like protein homolog 1 (C. elegans)_isoform CRA_b	154	Control
Q5M9H2	Acyl-Coenzyme A dehydrogenase_very long chain	29	Control
F1M609	Acyl-coenzyme A oxidase	26	Control
P25409	Alanine aminotransferase 1	73	Control
P11883	Aldehyde dehydrogenase_dimeric NADP-preferring	18	Control
P14046	Alpha-1-inhibitor 3	41	Control

P21571	ATP synthase-coupling factor 6_ mitochondrial	208	Control
D4A8B1	Bardet-Biedl syndrome 4	52	Control
D3ZCW2	BTB (POZ) domain containing 7 (Predicted)	53	Control
D4AC89	BTB domain-containing 11	34	Control
G3V9J2	Cadherin 7_ type 2	94	Control
Q5DWV2	Cadherin-7	94	Control
D3ZQR3	cAMP-regulated phosphoprotein 21	47	Control
Q63108	Carboxylesterase 1E	179	Control
P22734	Catechol O-methyltransferase	33	Control
A0A0G2JT93	Catenin (Cadherin associated protein)_ beta 1_ isoform CRA_a	60	Control
Q9WU82	Catenin beta-1	66	Control
D3ZPR0	Chromosome segregation 1-like	121	Control
Q5SGD7	Connector enhancer of kinase suppressor of ras 3	29	Control
Q6P4Z9	COP9 signalosome complex subunit 8	95	Control
P32551	Cytochrome b-c1 complex subunit 2_ mitochondrial	97	Control
A0A0G2K173	Cytochrome P450 1B1	50	Control
P20817	Cytochrome P450 4A14	152	Control
P20816	Cytochrome P450 4A2	130	Control
D3ZD73	DEAD-box helicase 6	33	Control
D4A2Z8	DEAH (Asp-Glu-Ala-His) box polypeptide 36 (Predicted)_ isoform CRA_a 1	38	Control
Q9EQ76	Dimethylaniline monooxygenase [N-oxide-forming] 3	103	Control
Q8K4B7	Dimethylaniline monooxygenase [N-oxide-forming] 4	19	Control
Q8K4C0	Dimethylaniline monooxygenase [N-oxide-forming] 5	125	Control
Q6AYS8	Estradiol 17-beta-dehydrogenase 11	66	Control
D3ZPD6	Fez family zinc finger 1	54	Control
M0R553	Flavin-containing monooxygenase	22	Control
D3ZT90	Glutaryl-CoA dehydrogenase	62	Control
Q6IE24	Inactive ubiquitin carboxyl-terminal hydrolase 54	23	Control
G3V824	Insulin-like growth factor 2 receptor	26	Control
G3V6G2	Integrator complex subunit 2	51	Control
Q9JK71	Membrane-associated guanylate kinase_ WW and PDZ domain-containing protein 3	37	Control
E9PTQ5	Methyl-CpG binding domain protein 5	36	Control
Q0D2L2	Mitochondrial ribosomal protein S22	79	Control
E9PT87	Myosin light chain kinase 3	33	Control
O54697	N-acetylated-alpha-linked acidic dipeptidase-like protein	45	Control
Q9ES54	Nuclear protein localization protein 4 homolog	37	Control
D3Z8Q6	Poly [ADP-ribose] polymerase	31	Control
Q5I0H9	Protein disulfide-isomerase A5	45	Control
A0A0G2JSZ5	Protein disulfide-isomerase A6	290	Control
Q5M9F1	RNA-binding protein 34	91	Control
Q6AY30	Saccharopine dehydrogenase-like oxidoreductase	58	Control
P11507	Sarcoplasmic/endoplasmic reticulum calcium ATPase 2	34	Control
Q5U311	Schlafen 8	37	Control
P12346	Serotransferrin	67	Control
Q498M9	Similar to glyceraldehyde-3-phosphate dehydrogenase	70	Control

G3V9Q6	Similar to putative TRAF and TNF receptor associated protein_ isoform CRA_c	79	Control
A0A096MJ9	Sodium/potassium-transporting ATPase subunit beta	112	Control
P07340	Sodium/potassium-transporting ATPase subunit beta-1	166	Control
D4A709	Tubulin_ gamma complex-associated protein 6	32	Control
Q3T1H5	Tyrosyl-DNA phosphodiesterase 2	79	Control
D3ZLQ8	Ubiquitinyl hydrolase 1	56	Control
D3ZEN2	Uncharacterized protein	70	Control
P45953	Very long-chain specific acyl-CoA dehydrogenase_ mitochondrial	32	Control
D3ZXZ1	Zinc finger protein 292	30	Control

^aIdentification is based on proteins ID from UniProt protein database, reviewed only (<http://www.uniprot.org/>).

^bProteins with expression significantly altered are organized according to the ratio.

*Indicates unique proteins in alphabetical order.

4- *Discussion*

4 DISCUSSION

In a recent study, we evaluated the effect of different F concentrations and exposure times on the profile of protein expression in the liver of rats. The most profound alterations were found in oxidative phosphorylation and cellular respiration, suggesting alterations in mitochondrial activity and energy metabolism (Pereira *et al.*, 2018). Due to this, in the present study, we decided to evaluate mitochondria instead of whole liver, in order to better focus on the alterations induced by F in energy metabolism. The extraction protocol we used is broadly employed to enrich the mitochondrial fraction (Frezza *et al.*, 2007). Despite most of the identified proteins are involved in Krebs cycle and oxidative phosphorylation, some cytosolic proteins, mainly involved in glycolysis and gluconeogenesis, were also identified. This suggests that our sample, despite enriched in mitochondrial fraction, was not completely devoid of cytosolic proteins, which is commonly reported in other studies (Li *et al.*, 2014; Morgenstern *et al.*, 2017; Chae *et al.*, 2018), and reflects the multi functionality of these proteins (Li *et al.*, 2014).

Since our main aim was to identify changes in the proteins involved in energy metabolism, the alterations found in proteins related to glycolysis, gluconeogenesis, urea cycle, β -oxidation, tricarboxylic acid cycle and electron transport chain, will be discussed separately. Considering that the energy metabolism is directly related to the production of ROS, alterations in antioxidant proteins will be also discussed, as well as calcium homeostasis.

For the bioinformatics analysis, most proteins with altered expression interacted with proteins involved in signaling pathways, such as 5'-AMP-activated protein kinase catalytic subunit alpha-1 (Prkaa1- catalytic subunit), 5'-AMP-activated protein kinase subunit beta 1 (Prkab1- non catalytic subunit) and Mitogen-activated protein kinase 3 (MAPK3). PKaa1 and Pkab1 play an essential role in the regulation of energy metabolism. Upon decreased intracellular ATP levels, AMPK activates energy-producing pathways and inhibits energy-consuming processes, such as biosynthesis of proteins, carbohydrates and lipids, as well as cell growth and proliferation. Prkaa1 protein also plays an important role in the control of oxidative stress (Foretz *et al.*, 2010; Wang *et al.*, 2010; Cambridge *et al.*, 2017) (UNIPROT). The presence of members of AMPK proteins among the interacting partners is in-line with the well-known effects of F on glycolysis (Sherer e Suttie, 1970) and oxidative stress (Sharma *et al.*, 2008). On the other hand, MAPK3 has the function of modulating connections between the

extracellular signaling pathways, thus controlling cascades of apoptosis, growth and proliferation (Dhillon *et al.*, 2007), which is also influenced by F (Otsuki *et al.*, 2005).

Glycolysis and Gluconeogenesis

Many of the proteins with altered expression are related to glycolysis and gluconeogenesis (Table 1). Among them are enzymes participating in both pathways that were reduced upon exposure to F (both concentrations) in the short-term, such as Fructose-bisphosphate aldose B (ALDOB), Glyceraldehyde 3 phosphate dehydrogenase (GAPDH), Phosphoglycerate Kinase 1 (PGK1), Alpha enolase and two isoforms of L-lactate dehydrogenase (LDH) (B and C chain). In addition, Pyruvate kinase (PKLR), a key protein in glycolysis was reduced upon exposure to the lowest dose of F in the short-term. Several studies have shown that F can bind to functional groups of amino acids around enzymatic active centers, inhibiting many enzymes among which are those involved in the glycolytic pathway, such as hexokinase, phosphofructokinase, pyruvate kinase, enolase and key enzymes for the generation of energy in the mitochondria (Sherer e Suttie, 1970; Shahed *et al.*, 1980; Vandercammen e Van Schaftingen, 1991; Adamek *et al.*, 2005; Barbier *et al.*, 2010). In the present study, however, we found that F reduced many glycolytic enzymes, which, associated with the ability of this ion to also inhibit some of these enzymes explains the effects of this ion in the energy metabolism (Pereira *et al.*, 2018), especially in bacteria (Hamilton, 1990). It is important to highlight that upon exposure to the lowest F concentration in the short-term, there was an increase in L-lactate dehydrogenase A chain (LDHA) (Figure 3B). Also LDHA was identified exclusively in the groups treated with 15 mg F/L (Figure 5A) and 50 mg F/L for 60 days, when compared with their respective controls. This trend of increase in LDHA might represent an attempt to find alternative routes for energy production (Kotoh *et al.*, 2011), as a consequence of glycolysis reduction, which has been reported in other studies (Dionizio, A. S. *et al.*, 2018; Malvezzi *et al.*, 2018).

The impairment in glycolysis in the presence of F is expected to activate alternative pathways for glucose production from non-carbohydrate precursors, such as gluconeogenesis (Nelson e Cox, 2012). In our study, the two major proteins related to gluconeogenesis, *Pyruvate Carboxylase* (PC) and *Fructose 1,6 Bisphosphatase* (FBP1), had their expressions changed in the F-treated groups. When the lowest dosage of F was given in the short term, the enzyme PC was upregulated (Table 1). This enzyme catalyzes the irreversible reaction of pyruvate to *Oxaloacetate* (OAA) that is later converted into *phosphoenolpyruvate* (PEP) by PEP carboxykinase (Nelson e Cox, 2012). PC also plays an important anaplerotic role in order to

replenish the supply of intermediates in the citric acid cycle (Jitrapakdee *et al.*, 2006; Jitrapakdee *et al.*, 2008). It should be noted that FBP1, a key enzyme in gluconeogenesis that is responsible for the irreversible conversion of fructose-1,6-phosphate to fructose-6-phosphate and is located downstream in respect to PC, was reduced upon exposure to F, regardless the dose. This indicates that glucose synthesis through gluconeogenesis might not be occurring, highlighting the anaplerotic role of PC upon exposure to F.

Another important protein with altered expression upon exposure to F was solute carrier family 2 facilitated glucose transporter member 2 (GLUT-2), which was reduced by F after the dose of 15 mgF/L in the period of 20 days, while in the period of 60 days it was exclusively identified in the control group in comparison to the group treated with 50 mgF/L (Table XX). This protein mediates the bidirectional transfer of glucose across the plasma membrane of hepatocytes and is also present in the kidney, small intestine and islets. Defects in the gene encoding this protein could alter glucose homeostasis (Zhou *et al.*, 2016). Some studies have reported changes in proteins related to glucose transport upon exposure to F (Lobo *et al.*, 2015; Pereira *et al.*, 2018). In the present study, the reduction of GLUT2 expression may suggest altered glucose transport, with impact on both glycolysis and gluconeogenesis.

It is noteworthy that in the shorter period, many proteins related to glycolysis and gluconeogenesis were reduced, while in the longer period, they were increased (Table 1), which might be related to cell adaptation to the presence of F (Dabrowska *et al.*, 2006; Pereira *et al.*, 2018), allowing the maintenance of the energy flow. These findings highlight the importance of considering not only the dose, but also the time of exposure to F when interpreting studies conducted under different methodological designs.

β-Oxidation

Mitochondrial β -oxidation is a catabolic pathway with the participation of several enzymes, where the oxidation of fatty acids generates acetyl-CoA, which is responsible for supplying energy to different tissues when glycogen stores are scarce and energy demand remains high (Leone *et al.*, 1999; Vishwanath, 2016). β -oxidation occurs in mitochondria and peroxisomes through four biochemical reactions: dehydrogenation, hydration, second dehydrogenation and thiolysis to generate Acetyl CoA, NADH^+ and FADH_2 from short, medium and long chain fatty acids (Kumar *et al.*, 2015; Adeva-Andany *et al.*, 2018). Fatty acids can come from different sources such as diet, *de novo* synthesis, release from the adipose tissue and different intracellular sites and the most important route for their oxidation occurs in mitochondria (Kunau *et al.*, 1995; Wanders *et al.*, 2010). To be oxidized, the fatty acid needs

to be converted into an activated form (acyl-CoA) that will be transported by the *Carnitine Palmitoyltransferase* (CPT1) and *mitochondrial carnitine / acylcarnitine carrier* (CACT) into the mitochondrial matrix, where it is oxidized to acetyl-CoA, which in turn can be completely degraded to CO₂ and H₂O through the TCA cycle and the electron transport chain (Wanders *et al.*, 2010; Ghimire e Dhamoon, 2018). Since liver plays a key role in energy homeostasis being able to balance the lipid metabolism beyond the energetic demands of the organism, FAO is stimulated during fasting in order to provide ketone bodies as an energy fuel. This generates large amounts of acetyl-CoA by β -oxidation, which may affect the oxidative capacity of the TCA cycle and thus the ketogenic pathway increases with subsequent generation of ketone bodies (Nasrin *et al.*, 2010; Ghimire e Dhamoon, 2018; Min *et al.*, 2018).

In the present study, several enzymes involved in different steps of FAO had change in expression that was distinct dependent on the dose of F and time of treatment. In general, the enzymes involved in β -oxidation were upregulated upon exposure to F, except for the dose of 50 mgF/L administered for 20 days that led to downregulation (Table 1). In the group treated with 15 mgF/L for 20 days, the increase in β -oxidation enzymes is probably trying to counteract the reduction of enzymes involved in glycolysis (Table 1), thus maintaining the energy flux. However, in the groups treated for 60 days, in general both enzymes involved in glycolysis and β -oxidation were increased, regardless the F dose. This increase in the long term was previously reported in the kidney (Xu *et al.*, 2005; Kobayashi *et al.*, 2009) and in the liver (Pereira *et al.*, 2013). According to Pereira *et al.* (2013), the proteomic analysis of rat livers revealed that after chronic exposure to F at different doses, Very Long Chain Acyl-CoA dehydrogenase was absent, suggesting an implication in the uptake of fatty acids, whereas 3-Ketoacyl-CoA mitochondrial thiolase was increased in the group receiving 50 mgF/L, indicating a stimulus in this pathway. Kobayashi *et al.* (2009) reported that upon exposure to 50 mgF/L dose, there was an increase in the expression of the enoyl coenzyme A hydratase in the kidney of rats, also suggesting an increase in β -oxidation.

Two important enzymes involved in the transport of fatty acids were altered. CPT1 was absent in the group that received 50 mgF/L for 20 days, while CACT was exclusively found in the groups treated with F for 20 days and at the group treated with the highest dose for 60 days. The presence of these transporters in the groups treated with F seems to be an attempt to keep the availability of fatty acids to be oxidized in order to maintain the energy flow (Vishwanath, 2016).

Enzymes involved in the production of ketone bodies were also altered. We observed an increase of the enzyme Hydroxymethylglutaryl-CoA synthase (HMG-CoA synthase) in the

groups that received F, regardless of the period (except for the 50 mgF/L group treated for 20 days), while Hydroxymethylglutaryl-CoA lyase (HMG- CoA lyase) was absent in the groups treated with F for 20 days. These results indicate that the production of acetoacetate might have been increased in the groups treated with F for 60 days, due to the increase in HMG-CoA synthase, one of the enzymes that limit the speed of the ketogenic pathway (Dashti e Ontko, 1979).

Urea Cycle

The urea cycle is an essential route for the excretion of nitrogen from the organism, occurring initially in the hepatic mitochondria and ending in the cytosol. When excessive amounts of amino acids are present and cannot be stored, they are therefore degraded (Devlin, 2011). This catabolic pathway includes transamination and oxidative deamination of amino acids, catalyzed by the enzyme glutamate dehydrogenase (GLDH) responsible for the release of ammonia, NADH and α -ketoglutarate (Morris, 2002). Part of the ammonia generated in this process is recycled and used in a variety of biosynthetic pathways where, under low energy conditions these products are beneficial. On the other hand, excess ammonia is excreted directly or converted into urea (Birkner E e Katowice R, 2000). Long-term changes in the activities of urea cycle enzymes may occur as response to alterations in the nitrogen flux of amino acids caused by changes in protein consumption, catabolism of endogenous proteins or when amino acids are diverted to anaplerotic reactions to be used in other energy-generating pathways (Morris, 2002). However, the mechanisms that may cause changes in the levels of liver enzymes involved in the urea cycle are far from being clarified.

Our results revealed that that mitochondrial Glutamate dehydrogenase (GLUD1) and mitochondrial Aspartate aminotransferase, also known as Aspartate transaminase (AST) showed increased expression or were exclusive in both periods and doses administered (except for the group that received the highest dose of F for 20 days), compared to the control group. This increased expression might be an attempt to keep the catabolism of amino acids, since it has been reported that exposure to F (10 and 30 mgF/L in the drinking water for 50 days) inhibited the activity of AST and GLDH (Grucka-Mamczar E, 1997). The activity of GLDH is typically higher in the liver than in the other organs, which can indicate a positive regulation of GLDH mRNA in response to metabolic adaptation, in order to avoid the release of toxic ammonia in the liver (Schmidt e Schmidt, 1963; Rothe *et al.*, 1994; Boon *et al.*, 1999; Spanaki e Plaitakis, 2012; Treberg *et al.*, 2014).

Regarding the enzymes involved in the urea cycle, Carbamoyl-phosphate synthase ammonia mitochondrial (CPS1) and Ornithine carbamoyltransferase_mitochondrial (OTC), first and second enzymes of the cycle were increased upon exposure to 15 mgF/L for 20 days, but decreased upon exposure to the highest F dose for the same period. Their increase upon exposure to the lowest dose might be an attempt to keep the urea cycle flow due to the reduction in downstream enzymes. Among these Argininosuccinate synthase (ASS1) was reduced in the groups treated for 20 days and exclusive in the groups treated for 60 days, regardless the dose of F. Reduction in the expression of ASS1 can increase cellular aspartate and this amino acid can be used in the biosynthesis of nucleotides and proteins (Kremer *et al.*, 2017), as well as be anaplerotically diverted to the Krebs cycle in order to maintain the energy flux. This is consistent with the reduction in several energetic pathways also with the increase in Malate dehydrogenase_mitochondrial (MDH2) upon exposure to F (regardless the dose) in the short-term. The anaplerotic conversion of Aspartate to oxaloacetate, coupled to increase in MDH2 can be an attempt to increase the rate of the TCA in the short-term. However, in the long-term ASS1 was identified exclusively in the F treated groups, which is consistent with the increase in the energy flux pathways, with no need to replenish the TCA intermediates. Consistent with the reduction in ASS1 in the short-period, there was also reduction in Arginase 1 (ARG1), regardless the dose of F (Table XX), as has been reported in the literature (Van Pilsum *et al.*, 1970; Pethe *et al.*, 2002). The decrease in ARG1, the last enzyme of the urea cycle, is consistent with the downregulation of upstream enzymes, indicating reduced flow of the urea cycle.

Tricarboxylic Acid Cycle

The tricarboxylic acid cycle (TCA) is an important pathway of catabolic and anabolic reactions. It is the common final route for many other pathways such as oxidation of carbohydrates, lipids, proteins and its main purpose is to oxidize Acetyl-CoA in the pathway or serve it as a precursor in the biosynthesis of fatty acids, cholesterol and ketone bodies (Akram, 2014). The cycle generates two important products that are the reductive compounds, NADH and FADH₂ that contain a large amount of energy stored in their molecules that will be oxidized in the electron transport chain (ETC) to generate ATP (Devlin, 2011; Nelson e Cox, 2012; Martinez-Reyes *et al.*, 2016).

For the group that received 15 mgF/L for 60 days, the enzymes identified were exclusive in respect to the control group (Table 1). Among them are *Isocitrate dehydrogenase NADP mitochondrial* (IDH2), responsible for reducing NADP⁺ to NADPH during the decarboxylation of isocitrate to form α -ketoglutarate, besides playing a critical role for

mitochondrial redox balance (Jo *et al.*, 2001; Reitman e Yan, 2010; Han *et al.*, 2018), as well as *Aconitate hydratase mitochondrial* (Aco2) that has the function of catalyzing the reversible interconversion of citrate into isocitrate, playing a critical role in NADPH homeostasis, acting as an oxidative stress biosensor, in addition to being used as an index of increased levels of ROS (Bulteau *et al.*, 2003; Chen *et al.*, 2005; Cantu *et al.*, 2009; Kim *et al.*, 2014). In addition, two isoforms of SuccinateCoA ligase had increased expression in the group that received the lowest dose of F in respect to the control group. On the other hand, they were absent in the group that received the highest dose of F for 20 days (Table 1). This enzyme plays an important role in maintaining mitochondrial DNA (mDNA) content, thus reduction in its levels may lead to mDNA depletion, which may result in mitochondrial imbalance, acting directly on mitochondrial complexes and causing bioenergetics deficiency (Miller *et al.*, 2011; Kacso *et al.*, 2016). The most relevant finding involving TCA cycle enzymes was the increase in Malate dehydrogenase mitochondrial (MDH2) upon exposure to F, except for the dose of 50 mgF/L administered for 20 days that led to downregulation (Table 1). MDH2 is a key enzyme in the TCA cycle, since one of its functions is the interconversion of malate to oxaloacetate (OAA) using the NAD/NADH coenzyme system. In addition, its activity is essential to control the redox system of the mitochondrial matrix, through its action in the regulation of TCA cycle (Goward e Nicholls, 1994; Nunes-Nesi *et al.*, 2005; Devlin, 2011; Linden *et al.*, 2016). In addition, the inhibition of the mitochondrial ETC is related to the inhibition of the activity of MDH2, since its cofactor is essential for the mitochondrial complexes and this inhibition can disturb mitochondrial respiration (Ban *et al.*, 2016). Thus, increase in this enzyme upon exposure to F is consistent with the increase in all energetic pathways observed in the present study, since TCA cycle connects all pathways involved in the energy generation.

Electron transport chain

The synthesis of ATP in eukaryotic cells happens mainly by the process called oxidative phosphorylation (OXPHOS) in the mitochondria. OXPHOS is a key unit in the mitochondria because it combines electron transport with cellular respiration and ATP synthesis. It is a process in which the electrons pass along several complexes and carrier molecules through the electron transport chain (ETC) (Saraste, 1999; Huttemann *et al.*, 2007). These electrons arise from the NADH and FADH₂, produced in glucose and/or fatty acids oxidation or in the TCA cycle, and then transferred to molecular oxygen through the complexes (Saraste, 1999). The OXPHOS consists of five organized complexes, of which three are considered proton pumps such as NADH dehydrogenase (complex I), complex bc₁ (complex III) and cytochrome c

oxidase (complex IV) that carry the protons and produce the proton gradient that is used by ATP synthase (complex V) to synthesize ATP from ADP and phosphate (Kadenbach *et al.*, 2010; Hroudova e Fisar, 2013). On the other hand, the succinate dehydrogenase complex (complex II) is a component related to the TCA cycle and participates in the ETC, where it is responsible for transferring electrons from the succinate to the ubiquinone, but does not contribute to the proton gradient, i.e., it only feeds the chain with electrons (Lee *et al.*, 2009; Kadenbach *et al.*, 2010; Douarre *et al.*, 2012; Guo *et al.*, 2013).

Due to the fact that mitochondria are the main producers of cellular energy, their regulation is important for cell viability under normal conditions and also during the periods of metabolic stress that can be caused, for example, upon exposure to F that may cause changes in the activities of mitochondrial enzymes, altering the mitochondrial respiration rate (Chauhan *et al.*, 2013). In our study, chronic administration of F produced significant changes in the OXPHOS. For the lowest dose of F, regardless the period of treatment, mitochondrial complexes I, II, III, IV and V had their expression increased or were exclusive in the treated groups (except for the ATP synthase subunit gamma mitochondrial that was reduced in the period of 20 days). On the other hand, upon exposure to the highest dose of F in the short period, the proteins were reduced or absent (except for the *ATP synthase subunit e mitochondrial* that was exclusively upon exposure to F), which was also observed in other energy pathways (Table 1). On the other hand, when administered the dose of 50 mgF/L for 60 days, the enzymes of complex I were increased, those of complex II were absent, the ones of complex III were reduced, and for complex IV, two isoforms of cytochrome oxidase were increased and two were reduced. A reduction was also observed for the complex V.

Studies investigating the effects of F in energy metabolism have shown a decrease in expression or inhibition of the activity of the mitochondrial complexes when F is administered (He *et al.*, 2012; Chauhan *et al.*, 2013; Fina *et al.*, 2014; Liang *et al.*, 2016; Panneerselvam *et al.*, 2017; Wang *et al.*, 2017). According to Fina *et al.* (2014), chronic or acute exposure to F in vitro inhibits complexes I and III of the ETC, which may result in an increase in the release of superoxide anions, thus increasing ROS levels. Chauhan *et al.* (2013) showed that the exposure to F modified the activity of mitochondrial enzymes of the intestine of rats, especially those of the mitochondrial complexes, which could damage the electron flow in the CTE, besides increasing nitric oxide levels during mitochondrial energy production.

Accentuated increase in the expression of the mitochondrial complexes can also increase the flow of mitochondrial superoxide and damage the mitochondrial DNA, besides contributing to the generation of oxidative stress and cellular damage (Alexeyev, 2009; Castro Mdel *et al.*,

2012; Voets *et al.*, 2012; Guo *et al.*, 2013). An important enzyme that was altered upon exposure to F was Cytochrome c-1 (CYC1) (Table 1). This enzyme is part of the complex III and is considered the major pathway for free radical generation within mitochondria. It regulates the mitochondrial function, besides being an important link between ETC and ATP production (Cela *et al.*, 2010; Roy Chowdhury e Banerji, 2018; Shimada *et al.*, 2018). Mitochondria can sense signs of metabolic damage caused by increase in ROS generation and compromise cells to apoptosis with the release of death-inducing factors, such as Cyt.C in the cytosol due to permeabilization of the mitochondrial outer membrane (Zhu *et al.*, 2012). However, in the present study, increased production of ROS that might have occurred in the groups treated with F (except for 50 mgF/L for 20 days) might have been counteracted by increase in the expression of antioxidant enzymes and antiapoptotic proteins, as will be described below.

Antioxidant system, Calcium homeostasis and Apoptosis

Approximately 90% of the O₂ consumed is used in OXPHOS to generate ATP. It is believed that the excessive release of electrons during the ETC is one of the causes of generation of ROS (K., 2018). The antioxidant defense enzymes such as glutathione peroxidase (GPx), glutathione transferases (GSTs), catalase (CAT), superoxide dismutase (SOD) and prohibitin (PHB) have the important function of maintaining ROS concentrations under balance in order to reduce the generation of oxidative stress (Sheu *et al.*, 2006).

In the present study, in general several isoforms of SOD, PHB, GPx, Peroxiredoxin (PRX) and CAT were reduced or absent when F was administered for 20 days, regardless the dose (Table 2, Tables S1-S4). However, when F was administered for 60 days, most of these enzymes were increased or exclusively identified in the group treated with 15 mgF/L, which indicates an attempt to counteract the generation of ROS due to the vigorous energy metabolism. Interestingly, exposure to 50 mgF/L for 20 days led to a reduction in energy metabolism, with probably low generation of ROS, what in turn led to reduction in antioxidant enzymes (Tables 1 and 2). However, in the long term, an increase in the energy metabolism was observed for this group, but most of the antioxidant enzymes did not present change in expression, while only two isoforms of PHB and SOD 1 were reduced. These findings suggest that studies employing longer periods of administration of F should be conducted to clarify the relationship between energy metabolism and antioxidant defense along time when higher doses of F are administered.

One reason for ROS generation is the accumulation or excessive influx of calcium (Ca^{2+}) into the mitochondria (K., 2018). The major intracellular source of Ca^{2+} in the cells is the endoplasmic reticulum (ER), while mitochondria are responsible for decoding the Ca^{2+} signals (Santo-Domingo e Demaurex, 2010). Normal levels of Ca^{2+} within the mitochondria can activate several dehydrogenase enzymes, thus raising the level of respiratory rate as well as proton extrusion and ATP production. However, Ca^{2+} overload may impair mitochondrial homeostasis, increase ROS and cause changes in the mitochondrial membrane potential, besides increasing the opening of the mitochondrial permeability transition pore complex (PTPC), resulting in apoptosis through the release of Cyt c (McCormack e Denton, 1989; Demaurex e Distelhorst, 2003; Kirichok *et al.*, 2004; Giorgi *et al.*, 2008; Santo-Domingo e Demaurex, 2010; Pereira *et al.*, 2018).

Some proteins that are involved in calcium homeostasis and apoptosis were altered upon exposure to F, such as: 1) isoforms of Voltage-dependent anion-selective channel protein (VDAC) that were increased in the lowest dose of F regardless the period; 2) isoforms of Calmodulin that were increased or exclusively identified in the treated groups in both periods (except for the highest dose of F for 20 days; 3) Mitochondrial proton/calcium exchanger that was increased in the lowest dose of F for 60 days; 4) Mitochondrial calcium uniporter (mCU) was identified exclusively in the control groups and 5) 3-ketoacyl-CoA thiolase_mitochondrial that was increased in all groups except in the group treated with 50 mgF/L for 20 days (Tables S1, S2, S3, S4). Calcium transporters are located throughout the mitochondrial membrane and also play an important role in the metabolic functions of this organelle transporting Ca^{2+} from the cytosol to the mitochondrial matrix (Santo-Domingo e Demaurex, 2010). The normal influx of Ca^{2+} occurs through the outer mitochondrial membrane and regulates the VDAC channel, but when in excess, it can cause the opening of this channel and alter the permeability, resulting in an increase in the release of Cyt c, which can lead to apoptosis (Juhaszova *et al.*, 2008).

Our results regarding the proteins related to Ca^{2+} homeostasis and apoptosis that were altered upon exposure to F indicate an attempt to maintain Ca^{2+} homeostasis and avoid apoptosis. In this sense, the increase in Calmodulin (increased or uniquely found in the F-treated groups) could help to inactivate mCU (expressed only in the control groups) activity thus preventing Ca^{2+} accumulation in the mitochondria (Moreau *et al.*, 2006; Santo-Domingo e Demaurex, 2010). On the other hand, VDAC was increased in the groups treated with 15 mgF/L. This protein may participate in the formation of the mitochondrial permeability transition pore (MPTP) complex, responsible for the release of mitochondrial products that triggers apoptosis (UNIPROT). The increase in VDAC was counteracted by the increase in 3-

ketoacyl-CoA thiolase_mitochondrial, an enzyme that links fatty acid metabolism to apoptosis, since it abolishes Cellular protein Bcl-2/adenovirus E1B 19 kD-interacting protein 3 (BNIP3)-mediated apoptosis by blocking mitochondrial damage (Cao *et al.*, 2008). Mitochondrial damage may result in an energetic catastrophe due to disruption of electron transport, oxidative phosphorylation and ATP production (Green e Reed, 1998) and lead to apoptosis (Lamy *et al.*, 2003). BNIP3 may initiate mitochondria-mediated apoptosis by inducing mitochondrial permeability transition pore (MPTP) opening to disrupt the proton electrochemical gradient across the inner mitochondrial membrane (Kubli *et al.*, 2007). The result is reduced mitochondrial respiration, excess generation of reactive oxygen species, and reduced ATP synthesis that can lead to cell death (Vande Velde *et al.*, 2000). Thus, increase in 3-ketoacyl-CoA thiolase_mitochondrial in the groups that were having vigorous energy flux might have prevented opening of the MPTP that could lead to apoptosis.

In summary, our results indicate that the direction of the energy flux depends on the dose of F and time of exposure. The dose of 15 mgF/L when administered for 20 days leads to a reduction in glycolysis, which is counteracted by an increase in β -oxidation, urea cycle and electron transport chain. At 60 days, however, an increase in all energetic pathways occurs. To compensate for the generation of ROS due to the vigorous state of energy production, there is a concomitant increase in antioxidant enzymes and in antiapoptotic proteins. However, the dose of 50 mgF/L when administered for 20 days reduces the enzymes involved in all energetic pathways. With less energy production there is probably less generation of ROS, what leads to reduction also in antioxidant enzymes. However, when 50 mgF/L is administered for 60 days, an increase in energy metabolism is observed, but the antioxidant enzymes in general do not present change in expression. Additional studies should be conducted employing longer periods of administration of high F doses, in order that the relationship between energy production and oxidative balance can be better understood. When analyzed in conjunction, these results suggest that the organism seems to adapt to the effects of F along time, activating pathways to reduce the toxicity of this ion. Ultimately, our findings corroborate the safety of the use of F for caries control.

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Annex

**Universidade de São Paulo
Faculdade de Odontologia de Bauru**

**Comissão de Ética no Ensino e Pesquisa em
Animais**

CEEPA-Proc. Nº 009/2017.

Bauru, 18 de agosto de 2017.

Senhora Professora,

Informamos que Projeto de Pesquisa denominado ***“Análise proteômica das mitocôndrias de fígados de ratos expostos à exposição crônica por fluoreto em dois períodos experimentais”*** tendo Vossa Senhoria como Pesquisador Responsável, que envolve a utilização de animais (roedores), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), foi analisado e considerado APROVADO em reunião ordinária da Comissão de Ética no Ensino e Pesquisa em Animais (CEEPA), realizada nesta data.

Vigência do projeto:	<i>Outubro/2017 a Março/2019</i>
Espécie/Linhagem:	<i>Serão utilizadas amostras de mitocôndrias de fígados de Rato Wistar, já coletadas em estudo anterior (Proc. CEEPA nº037/2011)</i>
Nº de animais:	<i>Não se aplica.</i>
Peso/Idade	<i>Não se aplica.</i>
Sexo:	<i>Não se aplica.</i>
Origem:	<i>Não se aplica.</i>

Esta CEEPA solicita que ao final da pesquisa seja enviado um Relatório com os resultados obtidos para análise ética e emissão de parecer final, o qual poderá ser utilizado para fins de publicação científica.

Atenciosamente,

Cássia Maria Fischer Rubira
Profª Drª Cássia Maria Fischer Rubira

Vice-Presidente da Comissão de Ética no Ensino e Pesquisa em Animais, em exercício

Profa. Dra. Marília Afonso Rabelo Buzalaf
Docente do Departamento de Ciências Biológicas

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
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<http://www.fob.usp.br>

Participação em encontro científico

Nome do evento: XIX Congress of the Brazilian Society for Cell Biology, São Paulo, Brazil, July 18th to 21th, 2018.

Apresentação de trabalho em forma de pôster: “Proteomic analysis of mitochondria of livers rats exposed to chronic fluoride exposure for 20 days “. **ARAUJO, T. T.**; SANCHES, C. C.; DIONIZIO, ALINE SALGADO; PEREIRA, HELOISA APARECIDA BARBOSA SILVA; BUZALAF, M.A.R.

Dexamethasone photodegradation and metabolite formation: an alternative to decrease the environmental impact. SANCHES, C. C.; PIPI, A. R.F.; XIMENES, V.F; SPAZZINI, F. C. R.; BERTOZO, L. C.; **ARAUJO, T. T.**; BUZALAF, CAMILA PERES.

Palestras assistidas durante o evento:

July 18th: Symp 1 – Cellular micro environments (Roger Chammas) / Advanced Microscopy (Hernandes Carvalho)/ Cell biology of neuroimmune interactions (Wilson Savino)/ Symp 5- Extracellular matrix (Marcelo Lamers)/ Symp 6 - RNA and cell Biology (Patrícia Coltri)/ Symp 7 – New genetic tools (Rodrigo Nunes da Fonseca)/ Symp 9 - Cell Migration (Claudia Mermelstein)/ Symp 10 - Cell proliferation & death.

July 19th : Cellular microenvironments (*Roger Chammas*; Luisa Iruela Arispe; Alexander Birbrair) / Regenerative medicine (*Silvy Stuchi Maria-Engler*; Rafael Linden; San W Won Han) / Advanced Microscopy (*Hernandes F Carvalho*; *Gustavo Menezes*; Carlos Lenz Cesar) / Cell biology neuroimmune interactions (Wilson Savino; Carmem Gottfried; Hartmut Wekerle) / Extracellular matrix (*Marcelo Lamers*; Sérgio Felisbino; Vanessa Freitas) / RNA and Cell Biology (*Patrícia P Coltri*; Kirill Afonin; Jorg Kobarg) / New genetic tools in developmental biology (*Rodrigo Nunes Fonseca*; Helena Araújo; Federico Brown) / Glia and disease (*Flavia Gomes*; Flávia Lima; Dora Brites).

July 20th

Cellular and molecular mechanisms of tumor progression (*Luiz Eurico Nasciutti*; Sabrina Battista; Thereza C Barja- Fidalgo; Martin H Bonamino) / DNA damage, epigenetics and IncRNA (*Enilza M Espreafico*; Miriam Galvonas Jasiulionis; TBA) / Cells & Organelles (*Luis Lamberti*; Martin Lowe; Ana Cláudia Torrecilhas) / Chromatin remodelling and DNA repair (*Silvia Batistuzzo de Medeiros*; Michelle Debatisse; Nicolas Hoch) / Cell migration and differentiation (*Manoel L Costa*; Cristiano Coutinho; Roberta Escaleira; Flavio Zolessi).

July 21st: Conf 13 Emer Ferro/ Conf 14 TBA/ Conf 15 Anselmo Moriscot SBBC 40 years/
Conf 16 André Pupo/ Conf 17 Glaucia Santelli/ Conf 18 Tiago Rodrigues.

Artigos publicados em 2018-2019

ARAUJO, T. T.; PEREIRA, H. A. B. S.; DIONIZIO, A. S.; SANCHEZ, C. C.; CARVALHO, T. S.; FERNANDES, M. S.; BUZALAF, M. A. R. Changes in energy metabolism induced by fluoride: insights from inside the mitochondria. **CHEMOSPHERE (UNDER REVIEW)**.

MALVEZZI, MARIA APARECIDA PEREIRA NUNES; PEREIRA, HELOISA APARECIDA BARBOSA SILVA; DIONIZIO, A. S.; **ARAUJO, T. T.**; BUZALAF, NATHALIA RABELO; SABINO-ARIAS, ISABELA TOMAZINI; FERNANDES, MILENI SILVA; GRIZZO, LARISSA TERCILIA; MAGALHÃES, ANA CAROLINA; BUZALAF, MARILIA AFONSO RABELO. Low-level fluoride exposure reduces glycemia in NOD mice. **ECOTOXICOLOGY AND ENVIRONMENTAL SAFETY**, v. 168, p. 198-204, 2019.

DIONIZIO, A. S.; PEREIRA, HELOISA APARECIDA BARBOSA SILVA; **ARAUJO, T. T.**; SABINO-ARIAS, ISABELA TOMAZINI; FERNANDES, MILENI SILVA; OLIVEIRA, KARINA APARECIDA; RAYMUNDO, FABIELLE SALES; CESTARI, TÂNIA MARY; NOGUEIRA, FERNANDO NEVES; CARVALHO, RUI ALBUQUERQUE; BUZALAF, MARÍLIA AFONSO RABELO. Effect of Duration of Exposure to Fluoride and Type of Diet on Lipid Parameters and De Novo Lipogenesis. **BIOLOGICAL TRACE ELEMENT RESEARCH**, v. 1, p. 1-15, 2018.

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