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Faculdade de Farmácia

Modulation of aquaporins gene expression by *n*-3 polyunsaturated fatty acids (PUFA) lipid structures in white and brown adipose tissue from hamsters

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Dissertação orientada pela Professora Doutora Graça Soveral e coorientada pela Doutora Paula Alexandra Lopes

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Abstract

The term "obesogenic environment" refers to the overabundance of food rich in energy coupled with reduced physical activity. The potential role of brown adipose tissue (BAT) in the pathophysiology of obesity or as a target for therapeutic intervention has been considered for decades, as brown adipocytes seem to be healthier than white. It is well-known that *n*-3 polyunsaturated fatty acids (PUFA), as eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3) have weight-reducing properties, with physiological activity depending directly on their molecular form, that is, as triacylglycerols (TAG) or ethyl esters (EE). In addition, aquaporins are membrane protein channels recognized as important players in controlling fat metabolism, but their differential expression in white adipose tissue (WAT) and BAT as well as their modulation by dietary PUFA has never been investigated. In this project, adipose tissue samples from subcutaneous and visceral WAT as well as from BAT, from hamsters fed on diets enriched with different *n*-3 PUFA (EPA and DHA) lipid structures (TAG and EE) as fish oil (FO) and fish oil ethyl esters (FO-EE), respectively were used to evaluate aquaporins mRNA expression and correlate them with markers of lipid metabolism. Linseed oil (LSO) was taken as the reference group. Our main findings are: (i) the differential characterization of aquaporins expression across WAT (subcutaneous and visceral) and BAT, which might reflect adipose tissue depot's own location and metabolic function; (ii) the modulation of aquaporins and lipid sensitive mediators transcriptional profile (adiponectin, leptin, glucose transporter type 4, peroxisome proliferator activated receptor α and peroxisome proliferator activated receptor γ) upon dependence of *n*-3 PUFA molecular structures (TAG or EE); (iii) the higher sensitivity of BAT than WAT to *n*-3 PUFA molecular structures; (iv) the upregulation of the majority of genes expression by FO diet, in opposition to LSO and FO-EE, highlighting the benefits of EPA and DHA combined as TAG; (v) among aquaporins, the aquaglyceroporin-7 stands out as the best option as therapeutic molecular target due to its conservative role across WAT and BAT.

Keywords: Aquaporins, *n*-3 PUFA, Brown adipose tissue, White adipose tissue, Hamster.

Resumo

O aumento do consumo de alimentos energéticos ricos em açúcar e gorduras saturadas, combinado com pouca atividade física, tem originado um aumento exponencial e preocupante da incidência da obesidade nas sociedades ocidentais ou ditas industrializadas. Várias são as consequências decorrentes da obesidade como é o caso de resistência à insulina, diabetes, dislipidemia, doenças cardiovasculares e cancro. Em Portugal as taxas de obesidade têm atingido valores preocupantes com cerca de 54% dos indivíduos diagnosticados com excesso de peso ou obesos. Diante deste problema de saúde mundial, a comunidade científica luta pela descoberta de novas terapias e alvos moleculares, que possam minimizar ou mesmo reverter esse cenário.

O tecido adiposo é um órgão multicelular que influencia profundamente a função de quase todos os órgãos do corpo humano através da produção de diversos metabolitos e de adipocinas. Os adipócitos denominados de células primárias do tecido adiposo têm como papel essencial manter a homeostase energética do organismo. O tecido adiposo classifica-se em tecido adiposo branco (WAT), tecido adiposo castanho (BAT) e tecido adiposo bege, com diferentes funções fisiológicas e localização. O WAT é composto por dois depósitos de gorduras distintos: o WAT subcutâneo (sWAT) e o WAT visceral (vWAT). A gordura subcutânea expande-se predominantemente por hiperplasia aparentando ser protetora dada a sua capacidade adipogénica aumentada, em contraste com a expansão da gordura visceral que ocorre principalmente por hipertrofia com grande infiltração de macrófagos e regulada por um elevado número de recetores de glicocorticóides e um reduzido número de recetores de insulina. Já a gordura castanha constitui um tecido metabolicamente responsável pela termogénese e pelo gasto do excesso de calorias. Em condições de excesso calórico, a gordura branca mantém a homeostase energética ao armazenar energia sob a forma de triacilgliceróis (TAG), enquanto em condições de necessidade energética os TAG são decompostos em glicerol e ácidos gordos livres para gerar energia sob a forma de adenosina trifosfato (ATP). Em contraste, o tecido adiposo castanho promove o gasto de energia através da oxidação da glicose e lípidos para gerar calor, através de uma respiração mitocondrial desacoplada mediada pela termogenina (UCP1) no processo chamado termogénese adaptativa.

A ingestão continuada de uma dieta rica em ácidos gordos saturados e ácidos gordos polinsaturados (PUFA) ómega-6 (*n*-6) e pobre em PUFA ómega-3 (*n*-3), denominada dieta ocidental, é considerada um dos principais fatores que promovem o desenvolvimento da obesidade. Em contraste, os ácidos gordos eicosapentaenóico (EPA, 20:5*n*-3) e

docosahexaenóico (DHA, 22:6*n*-3) possuem propriedades anti-adipogénicas. Acresce o facto de que a atividade fisiológica dos *n*-3 PUFA depende da sua estrutura molecular. Embora não consensual, parece existir uma diferença na biodisponibilidade aparente dos *n*-3 PUFA, uma vez que a forma de TAG apresenta maior biodisponiblidade do que a forma de ésteres etílicos (EE). A maioria dos suplementos alimentares ricos em EPA e DHA industrialmente disponíveis estão na forma de ésteres de etilo, o que pode dever ao custo reduzido da sua produção.

As aquaporinas (AQPs) são proteínas transmembranares que formam canais proteicos facilitando a passagem de água e pequenos solutos não carregados através das membranas celulares. De acordo com suas características de permeabilidade, as aquaporinas podem ser divididas em três subgrupos: aquaporinas clássicas ou ortodoxas (canais de água puros), aquagliceroporinas (canais permeados pela água e por glicerol, entre outros pequenos solutos) e as S-aquaporinas (de localização subcelular e permeabilidade ainda pouco conhecida). No tecido adiposo, o transporte alterado de glicerol mediado pela aquaporina-7 (AQP7) (uma aquagliceroporina) foi correlacionado positivamente com a acumulação de TAG nos adipócitos e com o aparecimento e desenvolvimento da obesidade. Mais recentemente, outras AQPs como a AQP3, AQP5, AQP9, AQP10 e a AQP11, foram também encontradas expressas no tecido adiposo. Contudo muito ainda há por descobrir em relação à função metabólica de cada uma AQP nos vários tipos de tecido adiposo.

A presente tese de mestrado tem como principal objetivo a determinação da expressão dos genes das aquaporinas *AQP3*, *AQP5* e *AQP7*, bem como de marcadores do metabolismo lipídico (adipocinas, adiponectina (*ADIPO*), leptina (*LEP*), transportador de glucose tipo 4 (*GLUT4*), recetores ativados por proliferadores de peroxissomas alfa e gama (*PPARα* e *PPARγ*) em três depósitos de gordura distintos, o branco (sWAT e vWAT) e o castanho (BAT) provenientes de hamsters alimentados com três dietas distintas enriquecidas em *n*-3 PUFA.

O modelo animal selecionado para este estudo foi o hamster por dois motivos principais: i) a existência de gordura castanha neste animal por força da hibernação e ii) o reconhecimento de que metabolismo lipídico no hamster é mais semelhante ao do humano do que o dos modelos convencionais de laboratório como o rato e o ratinho. Assim, 24 hamsters foram divididos em três grupos (com 8 animais por grupo) e alimentados com três diferentes dietas ricas em ácidos gordos polinsaturados *n*-3 de diferentes origens: óleo de linhaça (grupo LSO, rico em ácido alfa linolénico), óleo de peixe (grupo FO rico em EPA e DHA na forma estrutural de TAG) e óleo de peixe na forma de EE (grupo FO-EE rico em EPA e DHA na

forma de EE). A dieta LSO foi considerada a dieta controlo por não ter EPA nem DHA na sua composição. Ao invés apresenta o ácido gordo polinsaturado 18:3*n*-3, denominado alfa linolénico, percursor do EPA e DHA. A determinação da expressão relativa dos genes em estudo foi efetuada por PCR quantitativo em tempo real (RT-qPCR). Como dados complementares apresenta-se ainda nesta tese a caracterização dos grupos em estudo por meio do perfil plasmático bioquímico e de adipocinas bem como a composição em ácidos gordos das diferentes gorduras, branca subcutânea e branca visceral, como também castanha. Estes dados foram obtidos através de um projeto em curso na FMV-ULisboa e facultados generosamente para correlação com os resultados deste trabalho. Após análise e tratamento estatístico dos dados, as principais conclusões apresentam-se sumariadas nos seguintes pontos: (i) a caracterização diferencial da expressão das aquaporinas, *AQP3*, *AQP5* e *AQP7* nos depósitos de gordura WAT (subcutâneo e visceral) e BAT, o que certamente reflete a função metabólica do tecido adiposo em causa; (ii) a modulação do perfil de expressão das aquaporinas *AQP3*, *AQP5* e *AQP7* em função da estrutura molecular de *n*-3 PUFA (TAG ou EE) presente na dieta; (iii) a maior sensibilidade da gordura BAT (relativamente à WAT) às estruturas moleculares de *n*-3 PUFA presentes na dieta; (iv) o efeito positivo (com pequenas exceções) da dieta FO, em oposição à LSO e à FO-EE, que regulou positivamente a maioria dos genes, destacando os benefícios do EPA e DHA combinados em TAG; (v) entre as aquaporinas encontradas, a *AQP7* destaca-se como a melhor opção para alvo molecular terapêutico dado o seu papel conservado no tecido adiposo branco e castanho.

Palavras-chave: Aquaporinas, *n*-3 PUFA, Tecido adiposo castanho, Tecido adiposo branco, Hamster.

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List of abbreviations

AA	Arachidonic acid
ADIPO	Adiponectin
ALA	Alpha-linolenic acid
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AQP10	Aquaporin-10
AQP3	Aquaporin-3
AQP5	Aquaporin-5
AQP7	Aquaporin-7
AQP9	Aquaporin-9
AQPs	Aquaporins
AST	Aspartate aminotransferase
ATGL	Adipose triglyceride lipase
ATP	Adenosine triphosphate
BAT	Brown adipose tissue
cDNA	Complementary deoxyribonucleic acid
Cq	Quantification cycle
DHA	Docosahexaenoic acid (22:6*n*-3)
DNA	Deoxyribonucleic Acid
EE	Ethyl esters
EPA	Eicosapentaenoic acid (20:5*n*-3)
ETA	Eicosatetraenoic acid
FAME	Fatty acid methyl esters
FO	Fish oil
FO-EE	Fish oil ethyl esters
GADPH	Glyceraldehyde-3-phosphate dehydrogenase
GLUT4	Glucose transporter type 4
HDL	High density lipoproteins
LA	Linoleic acid
LDL	Low density lipoproteins
LEP	Leptin

LPL	Lipoprotein lipase
LSO	Linseed oil
mRNA	Messenger ribonucleic acid
MUFA	Monounsaturated fatty acid
ND	Not detected
NS	Not significantly different
PCA	Principal component analysis
PPARα	Peroxisome proliferator activated receptor alpha
PPARγ	Peroxisome proliferator activated receptor gamma
PUFA	Polyunsaturated fatty acids
QUICKI	Quantitative insulin sensitivity check index
r	Pearson's correlation coefficients
RNA	Ribonucleic acid
RNase	Ribonuclease
RPL27	Ribosomal protein L27
RT-qPCR	Real time quantitative polymerase chain reaction
SA	Stearidonic acid
SCD	Stearoyl-CoA desaturase
SE	Standard error
SFA	Saturated fatty acids
sWAT	Subcutaneous white adipose tissue
TAG	Triacylglycerols
TBHQ	Tertiary butyl hydroquinone
UCP1	Uncoupling protein 1
VLDL	Very low density lipoproteins
vWAT	Visceral white adipose tissue
WAT	White adipose tissue
β-actin	Beta actin
γ-GT	Gamma glutamyl transferase

1. Introduction

The most common adipose tissue disorder in humans is obesity, which can be defined as a chronic condition caused by excess energy intake relative to energy expenditure, resulting in a positive energy balance and weight gain (Spalding et al., 2008). The number of studies in the field of adipose tissue biology has increased exponentially since obesity is occurring at epidemic rates not only in developed countries, but also in developing countries (Swinburn et al., 2011). The increase of food consumption and fast food with high levels of sugar and saturated fats combined with a sedentary lifestyle has raised three-fold the obesity rates in industrialized societies.

According to the World Health Organization (WHO) in 2016, nearly 2 billion adults with 18 years and older are overweight, and of these more than 500 million are obese; in addition, 18% of children aged 5-19 are overweight or obese. This statistics naturally increases the risk for developing insulin resistance, type 2 diabetes, cardiovascular diseases, metabolic syndrome and certain immune metabolic dysfunctions (Van Gaal et al., 2006).

1.1 Adipose tissue

Adipose tissue is an extremely plastic organ capable of reduction, expansion or alteration according to appropriate stimulations. Studies published over the last two decades have established that adipose tissue acts not only as repository for excess nutrients but also as integrator and regulator of the balance between food intake and energy output. Adipose tissue is composed by adipocytes, vascular tissue, and immune cells (Berry et al., 2013). Consequently, according to its metabolic, cellular and endocrine functions, the adipose tissue is divided into two major types, the white adipose tissue (WAT) and the brown adipose tissue (BAT) with different functions. While white fat stores energy, brown fat expends it (Lowell & Flier, 1997). The current classification of adipose tissue includes a third category, the beige adipose tissue (brown-like-in-white), which has been detected in humans when stimulated by cold stress or β3-adrenoceptor agonists that mimic cold stress and shows thermogenic properties similar to BAT (Wu et al., 2012). The three types of adipose tissue have different morphology, distribution, gene expression, and function (Saely et al., 2012).

1.1.1 White adipose tissue

The WAT in mammals is the major form of adipose tissue. Its development has evolved as a physiological adaptation to preserve energy. Excess WAT is linked to obesity-

related health problems in nutritionally rich environments. WAT contains multiple cell types, mostly white adipocytes that have a single large cytoplasmatic lipid droplet with the nucleus located at the side of the cell (Figure 1A). Excess energy as triacylglycerols (TAG) is stored by white adipocytes, which provides a survival advantage in times of starvation.

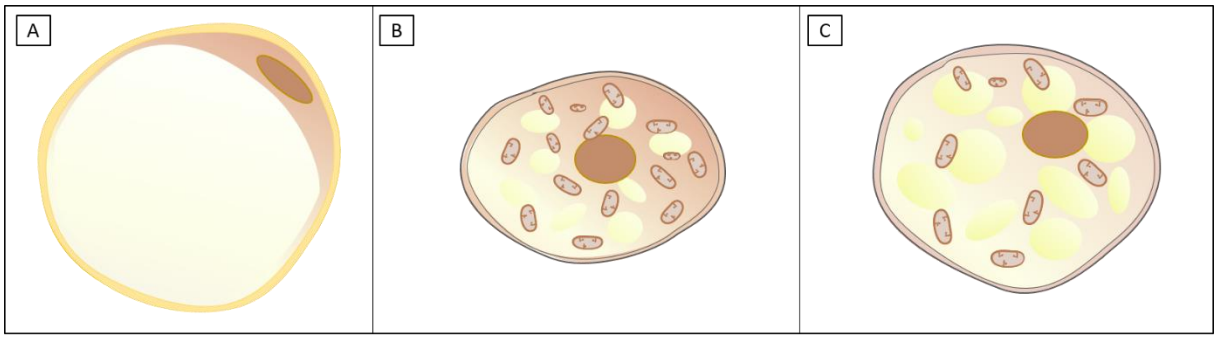

Figure 1. Main morphological characteristics of white, beige and brown adipocytes. **A**: White adipocytes are generally spherical with over 90% of the cell volume taken up by a single fat droplet. The few small mitochondria and the nucleus are compressed to the very edge of the white adipocyte. Mitochondria within white adipocytes are few and smaller. **B**: Brown adipocytes are smaller in overall size, have a polygonal shape, contain several small lipid droplets and high numbers of large mitochondria, which are responsible for the brown color. **C**: Beige adipocytes have mixed characteristics of both white and brown adipose cells.

Nowadays, it is known that WAT functions not only an energy reservoir but also as an insulator preventing heat loss and providing a physical protection to the organism. WAT is distributed throughout the body and is composed by two representative anatomical depots: subcutaneous WAT (sWAT) and visceral WAT (vWAT) (Figure 2). sWAT represents >80% of total adipose tissue in the body and is located inside the abdominal cavity and underneath the skin and scattered among skeletal muscles. The vWAT constitutes 6-20% of total body fat, with higher values in males than females (Seidell et al., 1988; Thomas et al., 1998; Ludescher et al., 2007; Haupt et al., 2010). It is located inside the peritoneum and distributed around internal organs (stomach, kidney, liver and intestine). Depending on the location, vWAT is subclassified into mesenteric, retroperitoneal, perigonadal, and omental (Park et al., 2014). Subcutaneous fat expands predominantly by hyperplasia (increase in the total fat cell number) and appears protective through enhanced adipogenic capacity, in contrast to visceral fat expansion that occurs mainly by hypertrophy (enlargement of the existing fat cells) with great infiltration of macrophages and regulated by a higher number of glucocorticoid receptors and a lower number of insulin receptors (Capurso & Capurso, 2012; Park et al., 2012; Laforest et al., 2015).

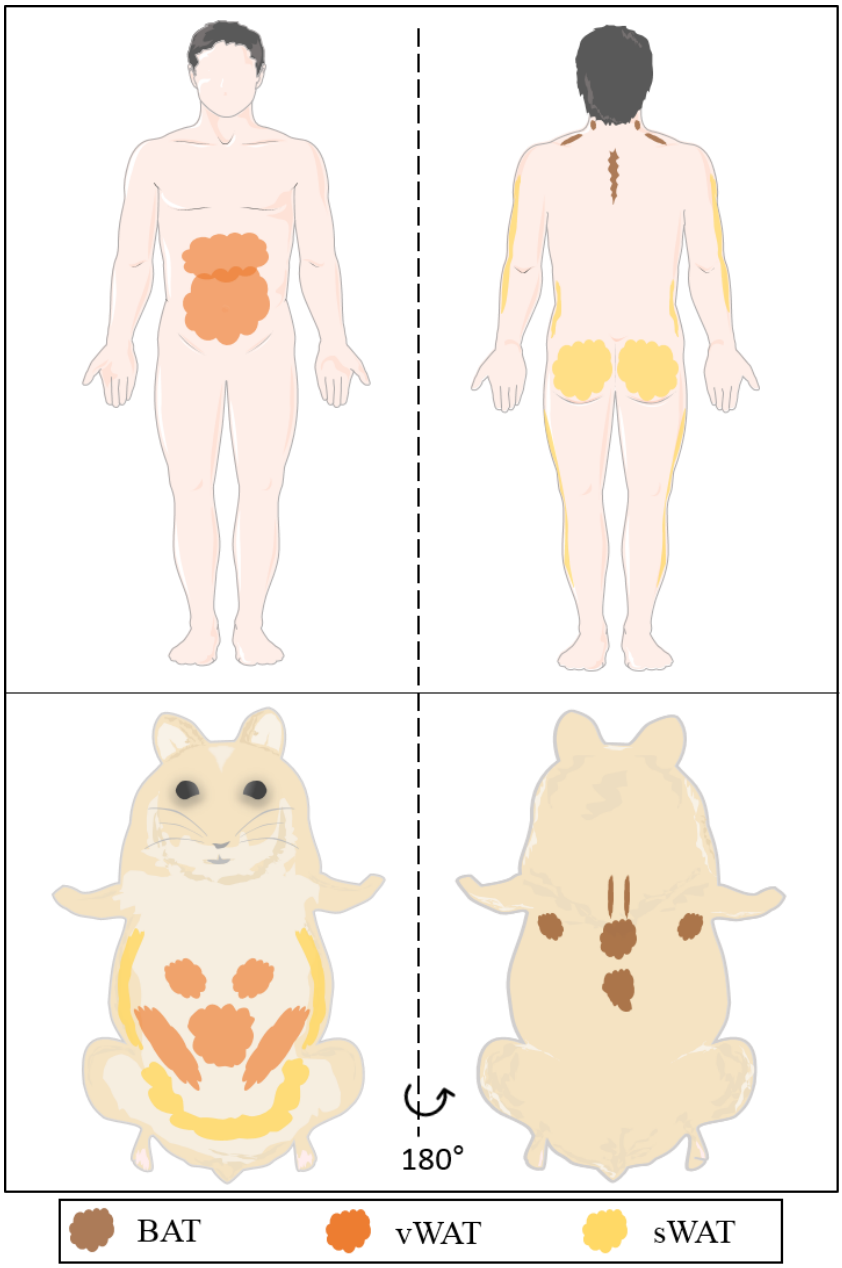

Figure 2. Locations of adipose tissue depots in adult human (A) and hamster (B).
Hamster has a major interscapular brown adipose tissue (BAT) depot, as illustrated. BAT is highly vascularized so that it can efficiently dissipate chemical energy as heat. White adipose tissue (WAT) is dispersed in various subcutaneous and intra-abdominal depots, and contains mostly white adipocytes. WAT is a major organ for the storage and release of energy (Saely et al., 2012).
vWAT, visceral white adipose tissue; sWAT, subcutaneous white adipose tissue; BAT, brown adipose tissue.

1.1.2 Brown adipose tissue

BAT is so-called because it is darkly pigmented owing to the high density of mitochondria rich in cytochromes. The lipid droplets in BAT are small and organized in

multilocular shape, and the nucleus appears roundish (Figure 1C). Until very recently, it was thought that adult humans do not have significant depots of brown fat, and that BAT exists only in hibernating mammals (like hamsters, see Figure 2) and newborns. This idea was changed when Nedergaard and Cannon, reported in 2007, that some adult human fat exhibits BAT characteristics (Nedergaard et al., 2007). Later on, this finding was confirmed by several scientific teams (Cypess et al., 2009; Saito et al., 2009; Virtanen et al., 2009; Zingaretti et al., 2009). Nowadays, it is known that BAT is located at the upper trunk, including the cervical, supraclavicular, paravertebral, and pericardial regions (Figure 2A). Healthy humans (around 70 Kg) have about 50 g of active BAT, approximately ~0.1% of body mass (Rothwell & Stock, 1979; van Marken Lichtenbelt & Schrauwen, 2011).

The thermogenic ability of brown adipocytes derives from the presence of uncoupling protein-1 (UCP1), an inner mitochondrial membrane protein that induces heat production by uncoupling respiration from ATP synthesis, a process termed non-shivering thermogenesis (Cannon & Nedergaard, 2004). Active BAT imports glucose and fatty acids to provide additional fuel for sustained thermogenesis (Labbe et al., 2015). Whether BAT has crucial functions beyond thermal regulation is also not clear, although BAT can secrete adipokines such as interleukin-6, fibroblast growth factor 21, and chemerin (Villarroya et al., 2013; Hansen et al., 2014). Although these adipokines are not BAT specific, their occurrence suggests that BAT has additional endocrine functions.

1.1.3 Beige adipose tissue

Beige adipocytes are found in various WAT depots and are especially prominent in sWAT. Beige fat cells develop in response to cold exposure and β-adrenergic stimulation (Harms & Seale, 2013; Ye et al., 2013). However, several addition factors capable of regulate fat browning have been discovered recently, such as irisin, fibroblast growth factor 21, follistatin, meteorin-like, among others (Braga et al., 2014; Rao et al., 2014; Zhang et al., 2014; Douris et al., 2015). Like brown adipocytes, beige cells have multilocular lipid droplets and densely packed UCP1-positive mitochondria (Figure 1B). Compared with brown adipocytes, beige adipocytes have more phenotypic flexibility, and can acquire a thermogenic or storage phenotype, depending on environmental cues (Wang & Seale, 2016). During basal state, beige fat displays unilocular morphology as white adipocyte, but upon stimulation, its appearance acquires features of intermediate morphology ultimately resulting in a transformation of stored fat into the small lipid droplets typical for brown adipocytes (Harms & Seale, 2013).

1.1.4 Adipose tissue biology

In situations of reduced energy expenditure or increased food intake which are characterized as positive energy balance, the mature adipocytes expand by hyperplasia (increase in number) and by hypertrophy (increase in size) changing their morphology to accommodate excess lipid due to increased free fatty acids (FFA) uptake and TAG synthesis. The extracellular matrix is adjusted to allow adipocyte enlargement by the action of proteases that hydrolyze the excess of collagen to allow adipose hypertrophy (Corvera & Gealekman, 2014). Besides of simple fat storage, adipose tissue is also a secretory and endocrine organ that produces hormones, such as adiponectin (ADIPO) and leptin (LEP), and has an important role in metabolic and vascular homeostasis, and inflammatory processes (Trayhurn & Beattie, 2001). ADIPO acts in adipocytes by increasing glucose transporter type 4 (GLUT4)-mediated glucose uptake while enhancing adipogenesis and adipocyte lipid storage (Fu et al., 2005). GLUT4 is a facilitative diffusion glucose transporter and is the major insulin-regulated glucose transporter in skeletal muscle, heart, and adipocytes. GLUT4 translocates from intracellular storage vesicles to the plasma membrane in adipocytes and muscle in response to increased insulin secretion (Shepherd & Kahn, 1999). Circulating levels of ADIPO increase while fat mass decreases. In turn the levels of LEP are often proportional to fat mass (Fantuzzi, 2005). LEP prevents lipogenesis by activating fatty acids β-oxidation (Cohen et al., 2005).

Facing weight gain and fat mass increase, the adipose tissue expands till a point where it no longer accumulates more fat. At this point, lipids start to deposit in other non-adipose tissues such as liver and muscle, leading to lipid-induced toxicity (lipotoxicity) and resulting in inflammation and insulin resistance (Hardy et al., 2012). vWAT is more closely related to liver through the portal vein than sWAT. This fact together with its diminished expansion capability, supports the increase risk of metabolic syndrome which is strongly associated with visceral obesity (Lee et al., 2012).

Recent data suggest that adipocyte mitochondria might play an important role in the development of obesity through defects in mitochondrial lipogenesis and lipolysis, regulation of adipocyte differentiation, apoptosis, efficiency of oxidative phosphorylation, and regulation of conversion of white adipocytes into brown-like adipocytes (De Pauw et al., 2009; Yin et al., 2014). Adipocyte differentiation appears to be controlled mainly by a major factor: the peroxisome proliferator-activated receptor gamma (PPARγ) (Rosen et al., 1999; Rosen & MacDougald, 2006). PPARγ is a regulator of all adipose tissue and is essential for WAT

development. Therefore, therapeutic intervention targeting these cellular processes could be a useful approach to reduce adiposity (Peschechera & Eckel, 2013).

1.3 Fatty acid general characterization

Fatty acids (FA) are carboxylic acids with long aliphatic tail chains. Natural FA commonly have a chain of 4 to 28 carbons, which according to its saturated state and structural and functional groups present can be classified as saturated fatty acids (SFA), monounsaturated fatty acids (MUFA) or polyunsaturated fatty acids (PUFA) (Crupi et al., 2013)

Fatty acids in the form of TAG are a principal source of energy and are also fundamental mediators of multiple signaling pathways and part of the structure and function of cell membranes (Kremmyda et al., 2011; Orsavova et al., 2015). On the other hand, some FA can be responsible for the expression of pro-inflammatory cytokines with negative effects for the human body (Kremmyda et al., 2011). The potential health effect of a specific fatty acid depends both on its structure and administration form (Bandarra et al., 2016). The level of saturation in a FA is responsible for the distinctive functions and interactions in all living beings. The more insaturated a fatty acid is, the more beneficial effects it has, being long-chain polyunsaturated fatty acid considered the most beneficial for lipid metabolism in humans (Grosso et al., 2014).

Fatty acid can have *cis* or *trans* configuration, based on the configuration of the double bonds, being the *trans* form a result of hydrogenation process from the food industry to create more stable solid fats from liquid oils (Estadella et al., 2013; Orsavova et al., 2015). *Trans* fats, however, are considered unhealthy, because they raise LDL cholesterol, lower HDL cholesterol, promote insulin resistance and are associated with systemic inflammation and endothelial dysfunction (Imran & Nadeem, 2015; Hinrichsen, 2016).

1.3.1 Saturated fatty acids

SFA are linear chain carboxylic acids that usually have 12 to 24 carbon atoms with no double bonds; instead SFA are saturated with hydrogen (Crupi et al., 2013). Palmitic acid (16:0) is the most common SFA and is usually found in palm oil (Hinrichsen, 2016). SFA can be also found in coconut oil (Orsavova et al., 2015), processed meat, milk, butter and other dairy products, salmon, egg yolks and chocolate (O'Sullivan et al., 2013; de Souza et al., 2015).

SFA are commonly associated with cardiovascular diseases, dyslipidemia, chronic inflammation, insulin resistance (Estadella et al., 2013), obesity and morphologic alterations (Campos-Silva et al., 2015), atherogenic potential and increased cholesterol levels (Hunter, 2001; Mensink et al., 2003), however other studies questioned these adverse effects, as no clear correlation was found between SFA and negative outcomes (Micha & Mozaffarian, 2010; Huth & Park, 2012; O'Sullivan et al., 2013; de Souza et al., 2015; Siri-Tarino et al., 2015).

1.3.2 Monounsaturated fatty acids

MUFA contain only a single double bond. An example of a common monounsaturated fatty acid is oleic acid (18:1*n*-9) that accounts for more than 92% of all MUFA consumed (Joris & Mensink, 2016). Oleic acid is mainly found in olive, rapeseed and sunflower oils, but MUFA are also generally found in red meat, whole fat milk products, nuts and canola oil (Lewinska et al., 2015; Orsavova et al., 2015).

The effects of MUFA are less studied than SFA and PUFA. Their positive role on cardiovascular disease is not very clear yet, though no harmful effects of MUFA-rich diets are known (Joris & Mensink, 2016). One study points out the beneficial effects of MUFA consumption along with fish oil (FO), rich in long-chain PUFA, on cardiovascular diseases, as MUFA can potentiate those benefit effects of FO (Kondreddy et al., 2016). Other studies state that oleic acid rich in MUFA appears to reduce low density lipoprotein (LDL) cholesterol level and also protects against oxidative modification of high-density lipoprotein cholesterol (HDL), but those effects are considered less beneficial when compared to PUFA role (Hunter, 2001; Lewinska et al., 2015).

1.3.3 Polyunsaturated fatty acids

PUFA contain two or more carbon-to-carbon double bonds in a hydrophobic hydrocarbon chain, not saturated with hydrogen atoms (Grosso et al., 2014). There are two main classes of PUFA, omega-3 (*n*-3) fatty acids and omega-6 (*n*-6) fatty acids, which differ in the position of their final carbon double bond and the fatty acid from which they are synthesized (Crupi et al., 2013). 18:3*n*-3 and 18:2*n*-6 are considered essential fatty acids since they play an important role in maintaining homeostatic conditions. Mammalian cells lack the desaturase enzymes required for their production, therefore these fatty acids must be obtained through the diet (Grosso et al., 2014).

The main sources of PUFA are mostly vegetable oils (soy, linseed, corn, sunflower, among others), fish flesh, liver and oil (codfish, salmon, tuna, sardines), and also seafood and marine algae (Crupi et al., 2013; Wibrand et al., 2013; Lewinska et al., 2015).

1.3.4 Biosynthesis and biological effects of PUFA

1.3.4.1 Omega-6 PUFA

The *n*-6 series derive from linoleic acid (LA, 18:2*n*-6) with the double bond starting at the sixth carbon atom from the end of the carbon chain. *n*-6 PUFA can be converted into arachidonic acid (AA, 20:4*n*-6) and then metabolized into the *n*-6 eicosanoids: lipoxins (LXs), prostaglandins (PGs), thromboxanes (TXs) and leukotrienes (LTs) (Grosso et al., 2014) (Figure 3).

1.3.4.2 Omega-3 PUFA

The *n*-3 series derive from a shorter-chained *n*-3 fatty acid, α-linolenic acid (ALA, 18:3*n*-3) with the double bond starting at the third carbon atom from the end of the carbon chain (Grosso et al., 2014) (Figure 3). *n*-3 synthesis forms the most important PUFA metabolites: eicosapentaenoic acid (EPA, 20:5*n*-3) and docosahexaenoic acid (DHA, 22:6*n*-3) (Das, 2003). The health effects of *n*-3 fatty acids come mostly from EPA and DHA (Molfino et al., 2014; Dyall, 2015), which will be detailed further.

1.3.5 PUFA synthesis

Essential fatty acids have important effects for human normal function but their full benefit comes from their long-chain metabolites that can be synthesized by a series of linked desaturation, chain elongation and β-oxidation reactions (Calder, 2012). Figure 3 shows the conversion scheme of *n*-6 and *n*-3 to their final metabolites and the enzymes involved in each step.

The synthesis of *n*-6 starts with the conversion of LA to γ-linolenic acid (18:3*n*-6) by the enzyme Δ6 desaturase. Synthesis of *n*-3 involves, starting with the conversion of ALA to stearidonic acid (SA, 18:4*n*-3) by Δ6-desaturase and SA to eicosatetraenoic acid (ETA, 20:4*n*-3) by the action of elongase. ETA is then converted to EPA by the action of the enzyme Δ5 desaturase. EPA can be converted to DHA by the action of an elongase, desaturase and β-oxidation reactions, with docosapenatenoic acid (DHA, 22:5*n*-3) being an intermediate in the pathway. ALA conversion to EPA is referred to be generally poor and as a result, DHA conversion is considered especially limited (Calder, 2012).

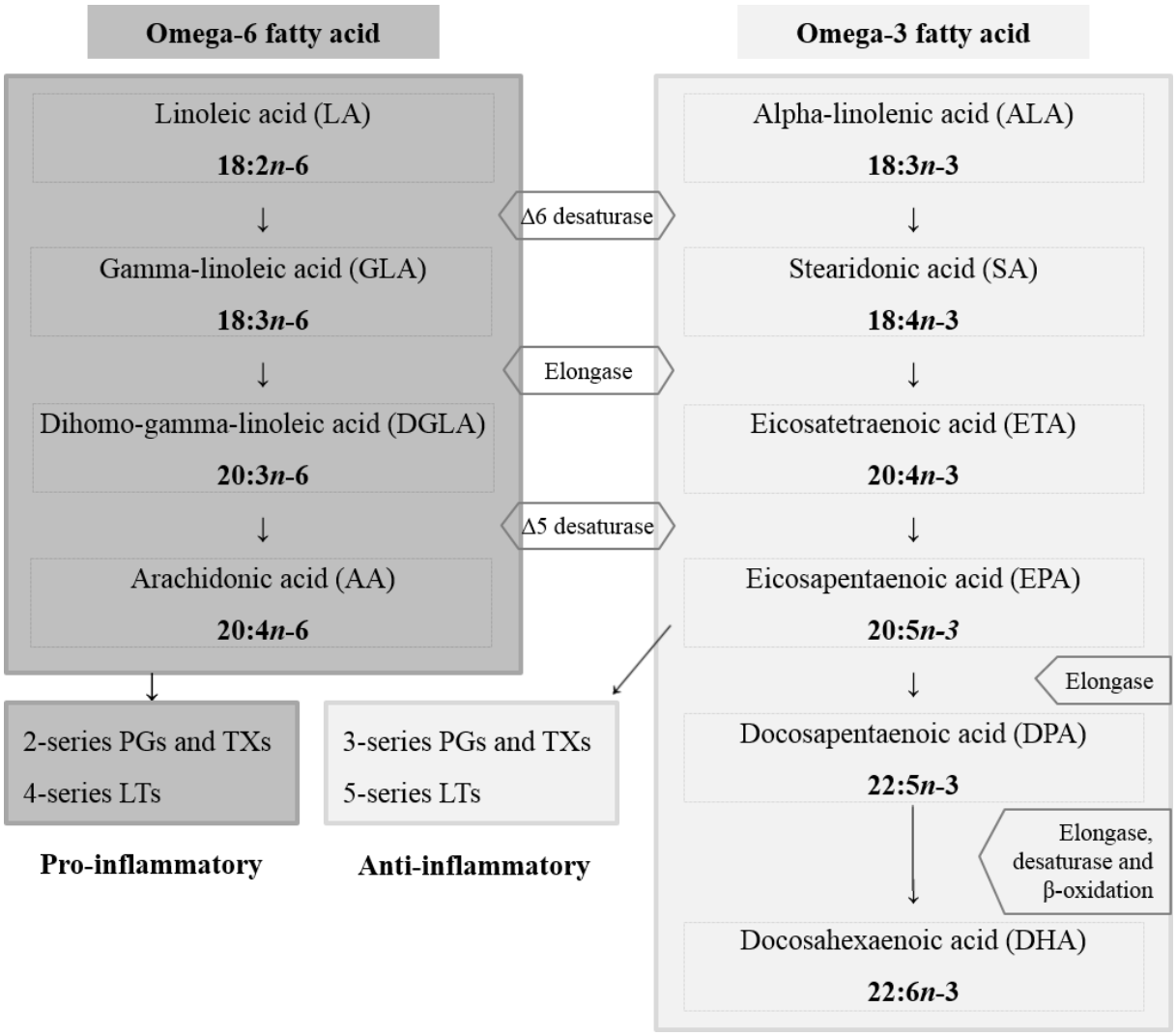

Figure 3. Biosynthesis of *n*-6 and *n*-3 fatty acids to their metabolites (adapted from (Grosso et al., 2014).

Both *n*-6 and *n*-3 series are metabolized by the same set of enzymes, desaturases and elongases. As a result, conversion of ALA to EPA competes with the conversion of LA to AA, being Δ6 desaturase the rate limiting factor in the pathway. Δ6 and Δ5 desaturases prefer *n*-3 to *n*-6, but their activities are regulated by a series of factors, such as nutritional status, hormones and end products feedback inhibition. While insulin activates Δ6 desaturase, glucose rich diets reduce it. Age also reduces the activity of Δ6 desaturase. A fat free diet and partial caloric restriction enhance Δ6 desaturase (Grosso et al., 2014).

n-3 derived eicosanoids are less active and their anti-inflammatory action can partially oppose the pro-inflammatory actions of *n*-6 eicosanoids. Therefore, to prevent a deregulation of inflammatory processes, a homeostatic balance between *n*-3 and *n*-6 fatty acid must be obtained (Grosso et al., 2014).

The concentration of *n*-3 in FO can be increased through ethylation. During this process, the glycerol backbone of TAG is removed from EPA and DHA and some of the shorter chain fatty acid are also taken out. DHA and EPA free fatty acids are then esterified to form ethyl esters (EE) (Figure 4). In EE, the fatty acids are esterified to an ethanol backbone, while in TAG the fatty acids are esterified to a glycerol alcohol backbone (Bezard et al., 1994). The majority of concentrated EPA and DHA products industrially available are in the ethyl ester form, which may be due to this lower cost of production.

Much remains to be discovered in relation to the effects of ethyl-ester -EPA and -DHA formulas compared to TAG-EPA and -DHA formulas.

1.3.6 Biological effects of *n*-6 and *n*-3

Since EPA competes with AA for enzymatic conversion, the increase of *n*-6 fatty acids will lead to a higher production of pro-inflammatory eicosanoids that cannot be neutralized by the anti-inflammatory effects of *n*-3 PUFA, which can be harmful to human body (Simopoulos, 2008). Lowering the ingestion of *n*-6 PUFA will increase the bioavailability of *n*-3 PUFA and, therefore, increase the concentration of EPA and DHA, which have beneficial anti-inflammatory properties (Taha et al., 2014).

Some of the *n*-6 eicosanoids, such as prostaglandins, thromboxanes, leukotrienes, hydroxyl fatty acids and lipoxins, are biologically active in very small quantities and, when formed in large amounts, can have a pro-inflammatory and pro-thrombotic action, which increases blood viscosity, causes vasospasm and vasoconstriction and decreases bleeding time (Simopoulos, 2008). An increase in *n*-6 eicosanoids can lead to the formation of thrombus, atheromas and to allergic and inflammatory disorders, potentiating pathological processes and chronic conditions, such as diabetes, cancer, obesity, autoimmune diseases and rheumatoid arthritis (Simopoulos, 2006). Pro-thrombotic and pro-inflammatory eicosanoids can also lead to cardiovascular events, elevate blood lipids and blood pressure levels (Khandelwal et al., 2013) and have a negative role on endothelial function, oxidative stress and even depression disorders (Husted & Bouzinova, 2016; Yang et al., 2016).

Long-chain PUFA are an important constituent of cell membranes and determine membranes properties, such as fluidity. Thus, the ratio between *n*-6 to *n*-3 fatty acid is important to avoid imbalance of membrane fluidity (Das & Fams, 2003). In 2008, a study from Griffin proposes that the *n*-6/*n*-3 ratio has no value as health risk. Instead, it can be used

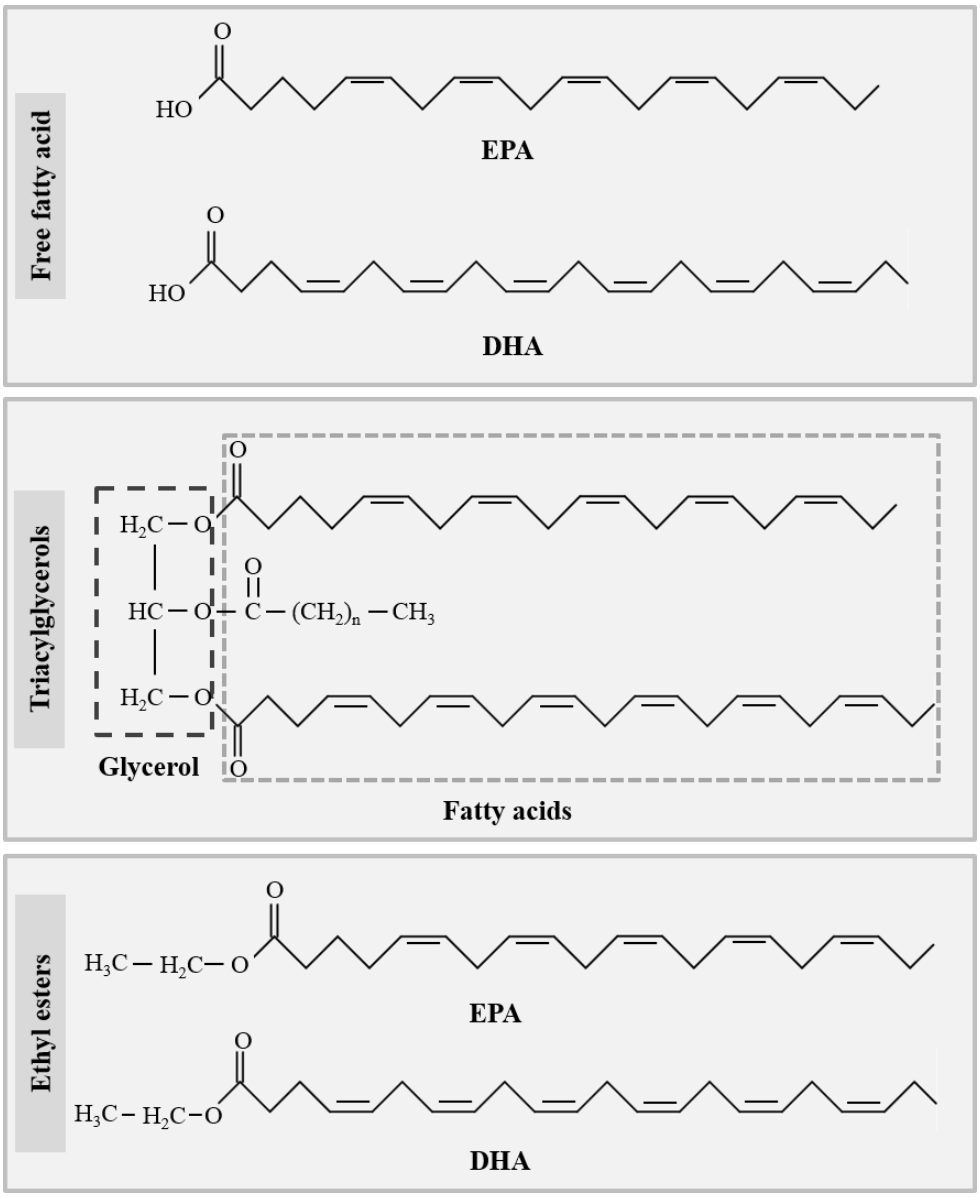

Figure 4. Structure of different forms of EPA (20:5*n*-3) and DHA (22:6*n*-3): free fatty acid (FFA, non-esterified fatty acids circulating in the plasma), triacylglycerols (TAG, an ester derived from glycerol and three fatty acids accumulated in the cytoplasm of adipose tissue (fish oil)), and ethyl esters (EE, only produced through chemical synthesis). EE form is created by reacting FFA with ethanol in a process called trans-esterification, when the glycerol backbone is removed of triacylglycerols fish oil resulting in FFA and a free glycerol molecule. The molecule of ethanol is then attached to each of the FFA (EPA and DHA) creating ethyl esters.

as a health indicator, but not determinant or prompter of disease (Griffin, 2008). Also, it states that the major contributors for health promotion are the *n*-3 metabolites, EPA and DHA, and the absolute amount of dietary PUFA are of relevance to the efficiency of the conversion of ALA to EPA and consequently of EPA to DHA. Therefore, a decrease in LA consumption

and an increase in ALA consumption will promote the endogenous synthesis of PUFA and increase health.

The therapeutic options for the treatment of obesity include dietary management, drug therapy, and bariatric surgery. Despite the wide range of treatments, the dietary intervention is the cornerstone of managing obesity and related morbidities. Numerous dietary supplements are being marketed as slimming aids. The efficacy of these food supplements has not been proven, yet they are sold. One such supplement is *n*-3 PUFA. Humans acquire *n*-3 PUFA from FO, which are obtained from the human diet or by consuming FO supplements or cod liver oil.

1.2 Aquaporins

Aquaporins are transmembrane protein channels that facilitate the transport of water and small solutes (such as glycerol) through the plasma membrane driven by osmotic or solute gradients (Agre, 2004; Carbrey & Agre, 2009). Adipose aquaporins (AQPs) are essential players in adipose tissue biology (Madeira et al., 2015b). In mammals, thirteen AQPs have been described. According to their primary sequence and permeability characteristics, aquaporins can be divided in three sub-groups: (i) orthodox or classical AQPs (AQP0, AQP1, AQP2, AQP4, AQP5, AQP6 and AQP8), primarily water selective facilitating water movement across cell membranes in response to osmotic or pressure gradients; (ii) aquaglyceroporins (AQP3, AQP7, AQP9 and AQP10) which transport some small uncharged solutes such as glycerol and urea in addition to water and; (iii) S-aquaporins or non-orthodox (AQP11 and AQP12) found mostly intracellularly, with lower sequence similarity to the other mammalian AQPs and with permeability features still uncertain (Figure 5) (Benga, 2012).

1.2.1 Aquaporin structure

AQPs are composed by around 320 amino acid residues with approximately 28kDa, architected in membranes as tetramers. Each monomer is formed by six transmembrane domains and behaves as an independent pore. The pore size is closely related to the channel selectivity (Figure 6). The three-dimensional structures of several AQPs enabled the conceptualization of a general structure, revealing the structural determinants that are essential for AQPs selectivity and extraordinary permeation rates. The atomic model of

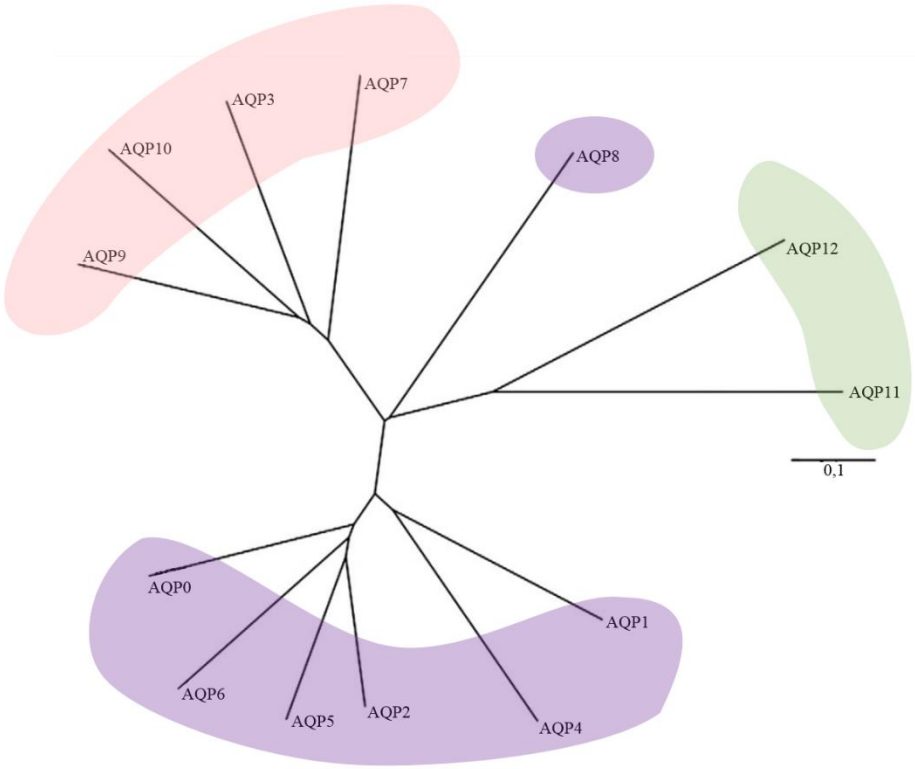

Figure 5. Phylogenetic tree of the human Aquaporin gene family (adapted from Ishibashi et al., 2014). Water permeable AQPs (AQP0, 1, 2, 4, 5, 6, 8) are shown in purple background. Glycerol permeable aquaglyceroporins (AQP3, 7, 9, 10) are in pink background. S-aquaporins (AQP11 and 12) are placed on the bottom right with green background. The scale bar represents genetic distance between homologues.

mammalian AQP1 derived from a 3.8 Å resolution potential map obtained by electron crystallography was the first atomic structure of a human membrane protein to be solved, and gave the first insight into AQP's water specificity (Murata et al., 2000). Medium and high-resolution structures of several AQPs belonging to different subfamilies have been determined from bacteria, yeast, plants, mammals, and others (Fu et al., 2000; Sui et al., 2001; Savage et al., 2003; Gonen et al., 2005; Tornroth-Horsefield et al., 2006; Horsefield et al., 2008; Fischer et al., 2009). More recently, molecular dynamics simulations have complemented the experimental data by providing the progression of the biomolecular system at atomic resolution (Hub et al., 2009). The reported structures have revealed that AQPs are grouped as homotetramers embedded in the bilayer, consisting of four independent monomers, each behaving as an independent channel (Figure 6) and sharing a conserved overall typical hourglass fold (Jung et al., 1994; Murata et al., 2000). Each monomer interacts with two of its neighbors, forming the tetramer central pore. It has been suggested that this pore, which is not involved in water conductance, may permeate gases and function as a gated

cation channel (Murata et al., 2000; Wang et al., 2007; Hub & de Groot, 2008; Wang & Tajkhorshid, 2010).

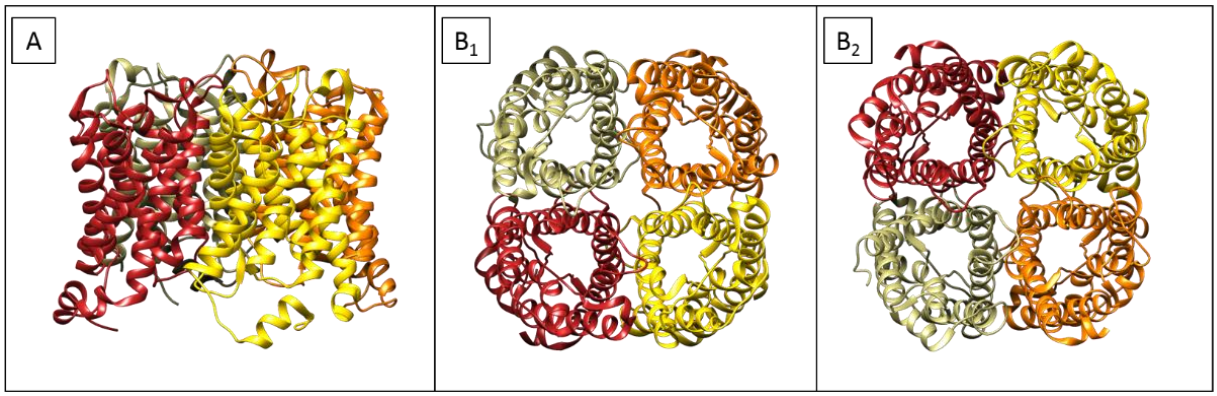

Figure 6. Tridimensional structure of hAQP5 (*Homo sapiens*). Homotetramer representation of the human AQP5 determined in 2008 by X-Ray Diffraction Method (2.0 Å resolution). Side (A), top (B_1) and down (B_2) view of the tetramer. Picture edited in UCSF Chimera software (PDB code: 3D9S).

1.2.2 Aquaporins in adipose tissue

Adipocytes hydrolyze TAG and rapidly liberate FFA and glycerol into the circulation. It is presumed that glycerol channels in adipocytes prevent acute rise in intracellular osmotic pressure when glycerol production is rapidly increased during lipolysis. Impaired glycerol transport through the aquaglyceroporin AQP7 has been correlated to TAG accumulation and obesity development (Madeira et al., 2015b). Recently, other AQPs, namely AQP3, AQP5, AQP9, AQP10 and AQP11 were reported in adipose tissue (Madeira et al., 2014; Madeira et al., 2015a; Madeira et al., 2015b; da Silva & Soveral, 2017), although their involvement in obesity mechanisms and divergent expression in BAT and WAT is still unclear. The first aquaporin that was detected in sWAT and vWAT was AQP7, which is the most representative glycerol channel.

AQP7 has been localized in a wide range of different tissues in both rodents and humans. The hypothesis on AQP7 as a facilitator of glycerol transport in adipose tissue was based on the assumption that AQP7 is expressed in the adipocyte plasma membrane (Fruhbeck, 2005; Fruhbeck et al., 2006; Maeda et al., 2008). The putative presence of other aquaglyceroporins in adipose tissue has been investigated with variable outcomes. Neither AQP3 or AQP9 mRNA were detected by northern blotting in WAT or BAT from mice (Kishida et al., 2000), and although not supported by all studies, similar results have been obtained for human WAT (Miranda et al., 2010; Lebeck, 2014). Some literature suggests the

presence of AQP10 in human WAT with localization only in the adipocyte; however, again this observation is disputed by others unable to detect AQP10 mRNA in adipose tissue (Miranda et al., 2010).

2. Aims

AQPs are emerging as important players in adipose tissue homeostasis and insulin response with possible implications in metabolic obesity-related disorders.

Taking advantage of a research project in development at FMV-ULisboa, we used fat tissues obtained from hamsters fed n-3 PUFA-enriched diets, and data from plasma lipid analysis obtained at FMV and from fatty acids determination in adipose tissue at IPMA, that were incorporated in this thesis to better correlate with gene expression results. In this study:

1) we postulated on the variability of aquaporins (*AQP3*, *AQP5* and *AQP7*) gene expression across subcutaneous WAT, visceral WAT and BAT, which might reflect adipose tissue depot's own location and metabolic function;

2) we hypothesized that different patterns of aquaporins expression exist between WAT (subcutaneous and visceral) and BAT when hamsters are fed on specific *n*-3 PUFA molecular structures: FO (rich in EPA and DHA in the TAG form) and FO-EE (rich in EPA and DHA in the EE form) *versus* linseed oil (LSO rich in ALA, 18:3*n*-3);

3) we further complemented this study with the transcriptional profile of lipid sensitive mediators, namely adiponectin (*ADIPO*), leptin (*LEP*), glucose transporter type 4 (*GLUT4*), peroxisome proliferator activated receptor alpha (*PPARα*) and peroxisome proliferator activated receptor gamma (*PPARγ*) across subcutaneous WAT, visceral WAT and BAT;

4) ultimately, the goal was to set novel molecular (such as aquaporins) and nutritional (such as *n*-3 PUFA) targets for developing new anti-obesity drugs and to translate mammals' successful evolutionary strategy, which is BAT, into a promising therapy to counteract obesity.

3. Materials and Methods

3.1 Ethics statement

The experimental procedures were reviewed by the Ethics Commission of CIISA/FMV and approved by the Animal Care Committee of the National Veterinary Authority (Direcção Geral de Alimentação e Veterinária, Portugal), following the appropriate European Union guidelines (2010/63/EU Directive). The experimental assay and euthanasia procedures were performed by project members certified for animal handling (category C by FELASA). In order to minimize animal suffering, the minimum number of animals and duration of observation were employed to gain reliable data.

3.2 Animals and diets

Besides having BAT in considerable amounts due to hibernation, the lipid metabolism of hamsters is more similar to humans than mice and rats (Yin et al., 2012; Dalboge et al., 2015), thus making it possible to fairly extrapolate results to humans.

Ten-week old Golden Syrian male hamsters were purchased from Charles River (Charles River Laboratories, L'Arbresle, France). Hamsters were housed one *per* cage, in standard cages (33 × 23 × 12 cm) under a 14/10 hour light/dark cycle schedule, synchronized with natural daylight, with controlled temperature of 20ºC-24ºC in a certified facility at the Faculty of Veterinary, University of Lisbon. During the first week, all animals were fed on a standard diet to minimize stress and stabilize all metabolic conditions. After this period, 24 hamsters were assigned to three body weight-matched groups, with eight animals each: the LSO group, rich in ALA (18:3*n*-3) without DHA or EPA, taken as the control group; the FO group, a commercial available oil rich in TAG with DHA and EPA and the FO-EE group. The final sum of DHA and EPA was identical across FO and FO-EE dietary treatments (Table1) (Bandarra et al., 2016; Lopes et al., 2017).

Throughout the trial, hamsters had free access to water and food. Body weight and feed intake were recorded twice a week. After 12 weeks of feeding trial, hamsters were fasted for 12 hours, weighted before and after the fasting period, and euthanized by a mechanical-physical method. Hamsters were placed in a chamber and anesthetized using a mixture of 20% of isofluorane in propylene glycol (v/v) for 30 seconds (Itah et al., 2004) followed by decapitation with a small animal guillotine, in certified ethical conditions to minimize animal suffering. Blood was collected into lithium heparin tubes and centrifuged at 1500*g*, room temperature, for 10 min, to isolate plasma, and stored at -80ºC for future biochemical

analysis. Subcutaneous and visceral WAT from hamsters pelvic and retroperitoneal anatomical regions, respectively as well as BAT from hamsters interscapular region were excised, weighed and stored at -80°C for subsequent fatty acid and gene expression determination.

3.3 Diets

Diets were manufactured by the Experimental Diets Unit from the University of Almeria. The proximate chemical composition of the diets was determined according to Association of Official Agricultural Chemists (AOAC, 1995), and fatty acid composition was assessed as described by Bandarra et al (2001) (Table 1). All diets were based on the AIN-93M formulation with modified lipid fractions. Each diet contained the following (g/100 g feed): casein (14.0), corn starch (46.6), maltodextrin (15.5), sucrose (10.0), cellulose (5.0), soybean oil (4.0), L-cystine (0.18), AIN-93 mineral mix (3.5), AIN-93M vitamin mix (1.0), choline bitartrate (0.25), and tert-butylhydroquinone (0.0008).

3.4 Determination of plasma metabolites and hormones

The concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-Cholesterol), low-density lipoprotein cholesterol (LDL-Cholesterol), TAG, glucose, creatinine, urea, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (γ-GT) in plasma were analyzed using standard diagnostic test kits from Roche Diagnostics (Mannheim, Germany) in a Modular Hitachi Analytical Systems (Roche Diagnostics). Very low-density lipoprotein cholesterol (VLDL-cholesterol) and total lipids were determined according to the formulas by Friedewald et al. (1972) and Covaci et al. (2006), respectively.

Plasma concentrations of the adipokines, LEP (R&D Systems, Minneapolis, USA) and ADIPO (R&D Systems) were measured by ELISA following the supplier recommendations. Insulin levels were measured in plasma using a commercial enzyme-linked immunosorbent assay kit (Mercodia, Uppsala, Sweden). The quantitative insulin sensitivity check index (QUICKI) was calculated using the inverse sum of the logarithms for fasting insulin and fasting glucose (Katz et al., 2000).

3.5 Determination of fatty acids in adipose tissue

The fatty acid methyl esters (FAME) were determined in the sWAT, vWAT and BAT, according to Bandarra et al. (Bandarra et al., 1997). All samples were lyophilized at -60ºC and

Table 1. Chemical composition (g/100g) and fatty acid profile (g/100g of total fatty acids) of the experimental diets.

	LSO	**FO**	**FO-EE**
Ingredients (g/100 g)			
Casein	14.0	14.0	14.0
Corn starch	46.6	46.6	46.6
Maltodextrin	15.5	15.5	15.5
Sucrose	10.0	10.0	10.0
Cellulose	5.0	5.0	5.0
Soybean oil	4.0	4.0	4.0
Linseed oil	0.335	-	-
Fish oil	-	0.796	-
Fish oil ethyl esters	-	-	0.796
L-Cysteine	0.18	0.18	0.18
Mineral AI*N*-93M mix	3.5	3.5	3.5
Vitamin AI*N*-93M mix	1.0	1.0	1.0
Choline bitartrate	0.25	0.25	0.25
TBHQ (antioxidant)	0.0008	0.0008	0.0008
Chemical composition (g/100 g)			
Gross energy (kcal/100 g)	337	341	345
Crude protein	11.5	11.1	11.5
Crude fat	1.3	1.8	1.8
Carbohydrates	69.7	70.2	70.7
Crude ash	2.9	2.9	2.9
Fatty acid profile (g/100 g of total fatty acids)			
10:0	4.43	1.87	2.09
12:0	4.76	3.00	2.30
14:0	0.300	1.00	1.40
16:0	10.2	12.0	11.4
16:1*n*-7	0.100	1.20	2.10
18:0	3.44	3.70	3.60
18:1*n*-9	22.1	22.9	23.4
18:2*n*-6	41.6	41.2	38.9
18:3*n*-3	7.88	5.80	5.54
18:4*n*-3	0.800	0.300	0.500
20:1*n*-9	ND	1.20	1.30
20:4*n*-3	ND	1.10	1.62
20:5*n*-3	ND	1.20	0.770
22:1*n*-11	ND	1.30	1.20
22:6*n*-3	ND	1.70	1.80

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group. TBHQ, tertiarybutylhydroquinone. ND, not detected.

2.0 hPa to a constant weight. FAME were obtained by adding 1 mL of anhydrous methanol, 0.5 mL of sodium methoxide (1 mol/L in methanol), swirling for 5 min, and 1 h reaction in the dark, according to Christie (Christie, 1989). The separation of the layer was enhanced through 10 min in ultrasonic bath and centrifuged (1500*g*, 5 min). The *n*-hexane layer was obtained, and the aqueous phase was re-extracted with 2.5 mL of *n*-hexane and centrifuged once more. FAME was concentrated to a final volume of 25 µL in *n*-hexane. 2 µL of sample was injected on a capillary DB-Wax capillary column (30 m, 0.25 mm internal diameter and 0.25 µm film thickness, J&W Scientific, Agilent, USA) in a Varian CP-3800 gas chromatograph (Varian, Palo Alto, USA) equipped with flame ionization detector. The temperatures were set at 250ºC in the injector and detector. Adequate separation was collected over 40 min, with 5 min at 180ºC, followed by an increase of 4ºC *per* minute until 220ºC, and kept at this temperature during 25 min. The identification of fatty acids was based on authentic standards and expressed as mol% of total fatty acids.

3.6 Adipocyte RNA extraction

Total RNA was isolated and purified from sWAT, vWAT and BAT using a Qiagen RNeasy lipid tissue mini kit (Qiagen, Hilden, Germany), and stored at -80ºC. To exclude possible DNA contamination, on-column DNA digestion with the RNase-free DNase set (Qiagen) was performed. All procedures were based on the manufacturer's protocol.

3.7 Measurement of RNA concentration and quality

The RNA concentration was determined spectrophotometrically at 260 nm using the NanoDrop1 ND-2000c (ThermoFisher Scientific, Waltham, USA). The ratios 260/280 nm and 260/230 nm were also determined to assess the purity of RNA samples and the presence of contaminants. Only samples that fulfill the established quality parameters (260/280 nm and 260/230 nm ratios were used as purity measurements for protein and solvent presence, respectively, considering ratios between 1.8-2.2) proceeded to complementary (cDNA) synthesis step.

3.8 cDNA synthesis

The synthesis of cDNA was performed using 750 ng of RNA and the retrotranscription reaction was carried out with a First-strand cDNA synthesis kit (NZYtech, Lisboa, Portugal) according to the manufacturer instructions. The final cDNA samples were diluted to 1:3 in water molecular biology grade (NZYtech) and stored at -20°C.

3.9 Primers design

Specific primers for real time quantitative PCR (RT-qPCR) were designed for seven genes (*AQP3, AQP5, AQP7, ADIPO, GLUT4, PPARα* and *PPARγ*). The DNA sequences of the golden hamster (*Mesocricetus auratus*) genes were obtained from GenBank (accession numbers listed in Table 2) and then submitted to the Primer3 software (http://primer3.ut.ee/) to generate the primers. Primer3 parameters were set in order to obtain the best pair of primers in size (20-27 bp), melting temperature (50-65ºC), % GC (50-60%) and product size range (75-200 bp). The characterization of the selected genes used in real-time quantitative PCR is described in Table 2.

3.10 RT-qPCR primers validation and optimization

To run a RT-qPCR assay is essential to validate each pair of primers to achieve accurate template quantification, each reaction must efficiently amplify a single product, and amplification efficiency must be independent of template concentration and amplification of other templates. The validation of primers was performed by assessing the efficiency (90-110%) and specificity (a single melting temperature) of the amplification. Primer optimization was performed, when the previous parameters were not fulfilled, by varying the annealing temperature (55-65ºC) and the primers forward and reverse concentration (100-300 nM) individually. Tables 3, 4 and 5 depict the optimized amplification conditions for the genes targeted in this project.

3.11 Real time Quantitative PCR (RT-qPCR)

Real time quantitative PCR was performed using PowerUp™ SYBR® Green Master Mix (Life Technologies, California, USA) which was prepared for a final reaction volume of 20 µL, using 3 µL of template cDNA, 2 µL of forward and reverse primers (Table 5.) and 3 µL of molecular biology grade water. The reaction was performed on a CFX96™ Real-Time PCR Detection System C1000 (BioRad, California, USA) consisting of an initial denaturation step at 95 °C for 3 minutes, 45 cycles of denaturation at 95 °C for 10 seconds and annealing/extension for 30 seconds; annealing temperatures are described in Table 3 and 4. A dissociation stage was added to determine the melting temperature (Tm) of a single nucleic acid target sequence as a quality and specificity measure. Relative expression levels were normalized to reference genes (*GAPDH* and *β-actin* for *AQP3, AQP5* and *AQP7; RPL27* for *ADIPO, LEP, GLUT4, PPARα* and *PPARγ*) and calculated using a variation of the Livak

Table 2. Gene specific primer sequences used for RT-qPCR.

Gene symbol	Full gene name	GenBank acession no.	Forward/reverse primers	Product size (bp)
AQP3	Aquaporin-3	XM_005078855.2	F: 5' CCAACAATGAGCTTATCGTCTCCG 3'	137
			R: 5' CAGAACACACACGATGAGGGAG 3'	
AQP5	Aquaporin-5	XM_005067227.2	F: 5' GGTGGTCATGGATCGGTTCAG 3'	100
			R: 5' GAAGAGCAGGTAGAAGTAGAGCAG 3'	
AQP7	Aquaporin-7	XM_013119574.1	F: 5' GCAGAGGGAGATGGTACGAGAG 3'	100
			R: 5' GTCTCCTAGAACCATATGAGCCAC 3'	
β-Actin	Beta actin	NM_001281595.1	F: 5' GCCAACCGTGAAAAGATGACC 3'	104
			R: 5' GTACGACCAGAGGCATACAGG 3'	
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase	DQ403055.1	F: 5' CAGTATGACTCTACCCATGGCAAG 3'	157
			R: 5' CAGTAGACTCCACAACATACTCGG 3'	
ADIPO	Adiponectin	GQ355976.1	F: 5' CTCTTCACCTTCGACCAGTATCAG 3'	139
			R: 5' CTGCATAGAGTCCACTGTAATCCC 3'	
RPL27	Ribosomal protein L27	XM_005070132.2	F: 5' CATGGGCAAGAAGAAAATCGCC 3'	155
			R: 5' GTTTCAGGGCTGGGTCTCTAAAG 3'	
LEP	Leptin	XM_005078071.2	F: 5' CCAAAACCCTCATCAAGACCA 3'	106
			R: 5' AGCCCAGGAATGAAGTCCAA 3'	
GLUT4	Solute carrier family 2 (facilitated glucose transporter), member 4	XM_005067520.2	F: 5' ATGGCTGTCGCTGGTTTCTC 3'	117
			R: 5' AAGCAGGAGGACGGCAAATA 3'	
PPARα	Peroxisome proliferator-activated receptor alpha	NM_001281543.1	F: 5' TGAGGAAGCCGTTCTGTGAC 3'	221
			R: 5' GGTGTCATCTGGATGGTTGC 3'	
PPARγ	Peroxisome proliferator-activated receptor gamma	XM_013110341.1	F: 5' GAGGGCGATCTTGACAGGAA 3'	139
			R: 5' GATGGCCACCTCTTTGCTCT 3'	

Table 3. Optimized conditions to quantify *AQP3, AQP5, AQP7, β-actin* and *GAPDH* mRNA expression levels by RT-qPCR.

	Temperature (ºC)	Duration	Cycles
Initial denaturation	95	3 minutes	1
Denaturing	95	10 seconds	45
Annealing/extension	59	30 seconds	

Table 4. Optimized conditions to quantify *ADIPO, LEP, GLUT4, PPARα, PPARγ* and *RPL27* mRNA expression levels by RT-qPCR.

	Temperature (ºC)	Duration	Cycles
Initial denaturation	95	3 minutes	1
Denaturing	95	10 seconds	45
Annealing/extension	62	30 seconds	

Table 5. Optimized concentration of forward and reverse primers for each target gene.

	Concentration (nM)	
	Forward	**Reverse**
AQP3	100	100
AQP5	300	300
AQP7	300	300
ADIPO	300	300
LEP	300	300
GLUT4	150	150
PPARα	300	300
PPARγ	300	300
β-actin	150	150
GAPDH	150	150
RPL27	300	300

method (Livak & Schmittgen, 2001), corrected for variation in amplification efficiency, as described by Fleige and Pfaffl (Fleige & Pfaffl, 2006; Fleige et al., 2006):

$$\text{Relative expression} = \frac{E^{CT}\,(Housekeeping\ gene)}{E^{CT}\,(Target\ gene)}\text{, E = 1 + efficiency of the reaction}$$

3.12 Statistical analyses

Statistical analyses were carried out with the Statistical Analysis System (SAS) software, version 9.4 (SAS Institute, Cary, NC, USA). All data were checked for normal distribution by Kolmogorov–Smirnov test and variance homogeneity using Proc MIXED of the SAS software package, with a model including the fat depot and diet as fixed effects and the repeated statement considering the group option to accommodate the variance heterogeneity. This analysis was followed by Tukey's multiple comparisons test. The Proc CORR method was used to obtain Pearson's correlation coefficients among genes expression and fatty acids. $P<0.05$ was considered to be statistically significant. Results are expressed as mean ± standard error (SE).

The principal component analysis (PCA) is a multivariate technique that reduces the dimensionality of data by transforming a number of related variables into a set of uncorrelated variables, which the maximum variability of the data was explained in a reduced variable set (Kadegowda et al., 2008), using the proportion of fatty acids common to all three tissues (subcutaneous WAT, visceral WAT and interscapular BAT) and gene expression levels. The

PCA was carried out using STATISTICA software, with the variables standardized to a mean of zero and a variance of one.

4. Results

4.1 Animal body composition

Body composition parameters of hamsters fed with different *n*-3 PUFA diets are shown in Table 6. The consumption of LSO, FO and FO-EE diets did not affect animal's daily feed intake, growth parameters or final body and tissue (liver and muscle) weights of hamsters ($P>0.05$).

4.2 Adipose tissue weight and plasma hormones

The variations in fat depots mass are illustrated in Figure 7. The subcutaneous WAT was the heaviest, the visceral WAT was the intermediate, and the interscapular BAT was the lightest ($P<0.05$), regardless the dietary group. Subcutaneous WAT, visceral WAT and interscapular BAT weights were unchanged by dietary treatments ($P>0.05$).

In line with this, Figure 8 presents the non-variations of plasma hormones across dietary groups, leptin ($P>0.05$; Figure 8A) and adiponectin ($P>0.05$; Figure 8B).

4.2 Plasma metabolites profile

Plasma metabolites from hamsters fed *n*-3 PUFA diets are also shown in Table 6. Hamsters fed FO-EE diet had lower HDL-Cholesterol, LDL-Cholesterol and total Cholesterol concentrations while FO had higher ($P<0.001$). VLDL-Cholesterol and TAG presented the lowest values in the LSO group than the other two experimental groups ($P<0.001$). Total lipids levels were different between the three dietary groups ($P<0.001$), being higher in FO group and lower in LSO group. FO and FO-EE diets reduced glucose concentration in comparison to the LSO dietary treatment ($P<0.001$). Moreover, the dietary treatments did not induce any significant change in insulin and insulin resistance marker (QUICKI) ($P>0.05$). Associated with renal function, creatinine levels were higher in LSO group and lower in FO-EE group ($P<0.001$). Urea parameter was lower in FO-EE in comparison with both FO and LSO groups ($P<0.001$). In relation to the hepatic markers, AST was decreased in the FO-EE group in relation to the other two groups ($P<0.001$). Higher concentrations of circulating ALT were found the LSO group and lower in the FO-EE group ($P<0.001$). ALP did not differ among the three groups ($P>0.05$). The gamma-glutamyl transferase (γ-GT) remained similar across dietary treatments ($P>0.05$).

Table 6. Body composition parameters and plasma metabolites.

	LSO		FO		FO-EE		*P* value	Significance
	Mean	SE	Mean	SE	Mean	SE		
Growth parameters and tissues weight (g)								
Initial body weight	137	3.93	136	4.24	142	3.89	0.497	NS
Final body weight	144	3.78	151	3.98	155	4.19	0.170	NS
Total body weight gain	7.15	2.96	14.5	2.81	12.5	3.60	0.210	NS
Daily feed intake	6.62	0.224	7.45	0.505	6.67	0.171	0.328	NS
Liver	4.54	0.401	5.45	0.291	5.12	0.269	0.205	NS
Muscle *(Longissimus dorsi)*	2.71	0.074	2.91	0.161	2.83	0.290	0.545	NS
Subcutaneous white adipose tissue	2.31	0.456	3.49	0.718	3.26	0.531	0.270	NS
Visceral white adipose tissue	1.60	0.167	2.07	0.137	2.18	0.100	0.162	NS
Interscapular brown adipose tissue	0.397	0.021	0.446	0.025	0.487	0.035	0.082	NS
Plasma biochemistry profile								
Total Cholesterol (mg/L)	126^{b}	2.70	144^{a}	3.45	113^{c}	2.71	<.001	***
HDL-Cholesterol (mg/L)	81.8^{b}	1.72	92.9^{a}	2.33	72.1^{c}	1.74	<.001	***
LDL-Cholesterol (mg/L)	24.9^{b}	0.581	28.1^{a}	1.01	22.1^{c}	0.515	<.001	***
VLDL-Cholesterol (mg/L) †	16.0^{b}	0.474	25.5^{a}	0.727	25.9^{a}	0.681	<.001	***
LDL-C/HDL-C	0.304	0.002	0.303	0.007	0.307	0.005	0.852	NS
TAG (mg/L)	80.1^{b}	2.37	127.4^{a}	3.64	129.3^{a}	3.41	<.001	***
Total lipids (mg/L) ‡	482^{c}	6.55	565^{a}	9.07	505^{b}	4.87	<.001	***
Glucose (mg/L)	98.4^{a}	2.49	78.9^{b}	1.45	84.6^{b}	2.46	<.001	***
Insulin (pg/mL)	341	78.0	449	122	438	46.5	0.437	NS
QUICKI (mmol/L × mU/mL) §	0.336	0.010	0.336	0.009	0.342	0.010	0.879	NS
Creatinine (mg/L)	0.355^{a}	0.014	0.303^{b}	0.006	0.221^{c}	0.013	<.001	***
Urea (mg/L)	40.8^{a}	1.33	38.7^{a}	1.21	29.8^{b}	0.796	<.001	***
Plasma hepatic markers								
AST (U/L)	101^{a}	2.92	104^{a}	2.53	72.4^{b}	1.73	<.001	***
ALT (U/L)	105^{a}	2.39	61.9^{b}	1.70	51.1^{c}	1.30	<.001	***
ALP (U/L)	105	2.82	94.7	3.09	100	3.01	0.075	NS
γ-GT (U/L)	4.25	0.620	4.57	0.649	5.63	0.706	0.342	NS

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group. *n*=8 *per* group. Statistical significance: NS = not significantly different, *P*>0.05; *, *P*<0.05; **, *P*<0.01; ***, *P*<0.001. Values are means ± SE. ^{a,b,c} Means in the same row with different superscripts are significantly different (Tukey's *post hoc*, $P < 0.05$).

†VLDL-cholesterol = 1/5 [triacylglycerols].

‡Total lipids = [total cholesterol] × 1.12 + [triacylglycerols] × 1.33 + 148.

§QUICKI, quantitative insulin sensitivity check index = 1 / (log fasting plasma glucose + fasting plasma insulin).

4.3 Fatty acid profile in subcutaneous white adipose tissue

The fatty acid profile of sWAT across dietary groups is shown in Table 7. The distribution pattern of the main fatty acid classes across dietary treatments showed a highest occurrence of MUFA (54%), followed by total PUFA (23 to 24%), SFA (22 to 24%), and lastly *n*-6 PUFA with a prevalence around 20 to 22%. SFA were unchanged under this experimental design (*P*>0.05), due to the non-variations of the main SFA, 14:0, 15:0, 16:0, 17:0, 18:0 and 19:0 (*P*>0.05). In a similar trend, the MUFA sum presented no differences (*P*>0.05) among groups, although small changes were found for the 20:1*n*-9 (*P*<0.05),

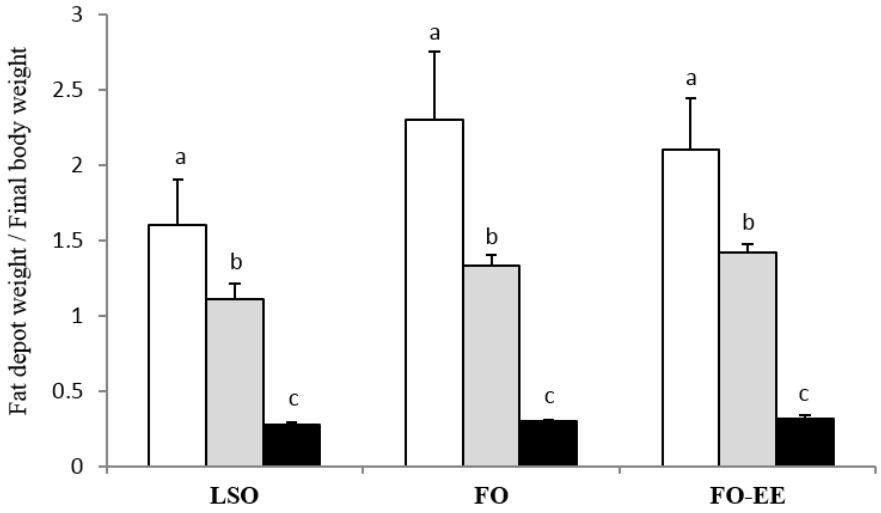

Figure 7. Subcutaneous WAT (white bars), visceral WAT (gray bars) and interscapular BAT (black bars) weight from hamsters fed on LSO, FO and FO-EE diets. Means (± SE) with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

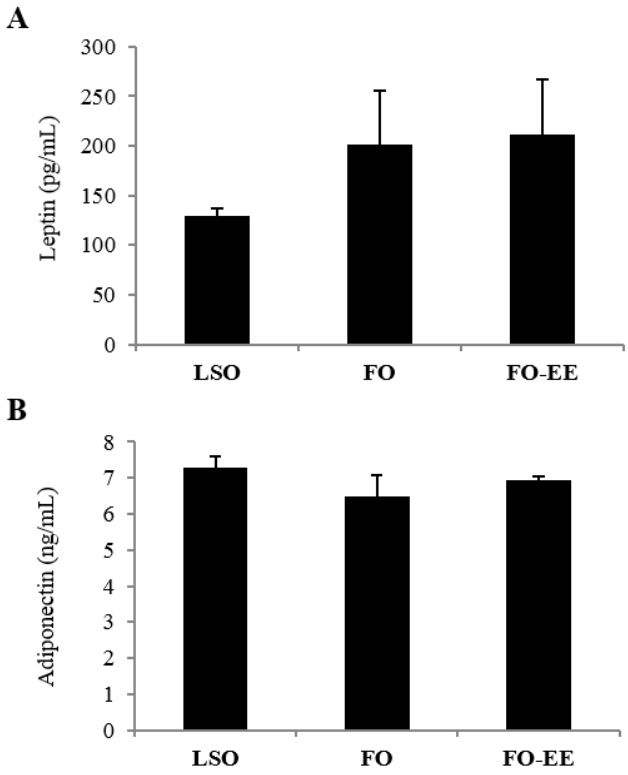

Figure 8. Plasma hormones (means ± SE), leptin (A) and adiponectin (B) from hamsters fed on LSO, FO and FO-EE diets.

16:1*n*-7 ($P<0.05$), and 16:1*n*-9 ($P<0.01$) fatty acids. Even if total PUFA did not change ($P>0.05$), the *n*-3 PUFA sum was increased in the LSO group in comparison to the others ($P<0.001$), at the expenses of ALA (18:3*n*-3) ($P<0.001$). EPA and DHA were not found. For

the *n*-6 PUFA sum, the non-variations observed reflect the ones of the LA (18:2*n*-6) ($P>0.05$). AA (20:4*n*-6), the most prevalent PUFA from the *n*-6 family was not found in any dietary treatment. While the stearoyl-CoA desaturase (SCD) ratio presented similar values across dietary groups ($P>0.05$), the *n*-3/*n*-6 ratio was increased in hamsters fed LSO diet relative to FO and FO-EE ($P<0.001$).

4.4 Fatty acid profile in visceral white adipose tissue

The fatty acid composition of vWAT is presented in Table 8. The distribution pattern of the main fatty acid classes across dietary groups showed a highest occurrence of MUFA (51 to 52%), followed by SFA (26 to 27%), closely followed by total PUFA (21 to 23%), and lastly by *n*-6 PUFA (19 to 20%). The main SFA, 14:0, 15:0, 16:0, 17:0, 18:0 and 19:0, as well as total SFA, presented no variations across dietary groups ($P>0.05$). The sum of MUFA did not vary ($P>0.05$), but 18:1*n*-9 was identically lower in FO and FO-EE but higher in LSO ($P<0.05$). Moreover, the 16:1*n*-7 percentage was higher in the FO and FO-EE groups when compared to the LSO group ($P<0.001$). 16:1*n*-9 was higher in the LSO and lower in the FO and FO-EE dietary groups ($P<0.05$). In turn, higher percentages of the 20:1*n*-9 were observed in the FO and FO-EE groups whereas lower percentages were observed in the LSO ($P<0.001$). Total and *n*-6 PUFA percentages presented no-variations across dietary groups ($P>0.05$), whereas *n*-3 PUFA sum was higher in the LSO group ($P<0.001$) at the expenses of ALA ($P<0.001$). In resemblance to the sWAT, AA was not found in any dietary treatment. EPA and DHA were, once again, not found in WAT. SCD ratio did not vary ($P>0.05$). The *n*-3/*n*-6 ratio reached the highest value in the LSO being the values similar for FO and FO-EE ($P<0.001$) groups.

4.5 Fatty acid profile in brown adipose tissue

The fatty acid profile of interscapular BAT is presented in Table 9. The distribution pattern of the main fatty acid classes across dietary groups showed a highest occurrence of MUFA (48 to 49%), followed by SFA (26 to 29%) and total PUFA (22 to 25%), and lastly by *n*-6 PUFA (20 to 22%). In opposition to the large variations described previously for visceral white fat, interscapular BAT presented fewer changes for fatty acids deposition across dietary treatments. The SFA sum was higher in FO and FO-EE groups relative to LSO ($P<0.05$). Except for 16:0 that reached the highest percentage in the FO and FO-EE ($P<0.05$) diets relative to LSO, no additional variations were found for the remaining SFA ($P>0.05$).

Table 7. Fatty acid composition (mol% of total fatty acids) of subcutaneous white adipose tissue.

	LSO		FO		FO-EE		*P* value	Significance
	Mean	SE	Mean	SE	Mean	SE		
11:0	0.078	0.011	0.090	0.020	0.066	0.015	0.611	NS
14:0	1.36	0.128	1.65	0.199	1.31	0.148	0.367	NS
15:0	0.554	0.047	0.572	0.040	0.494	0.045	0.434	NS
16:0	18.6	0.568	19.6	0.780	18.5	0.748	0.532	NS
16:1*n*-9	0.622^{a}	0.026	0.442^{b}	0.062	0.517^{b}	0.015	0.004	**
16:1*n*-7	9.31^{b}	0.397	12.9^{a}	1.14	$10.7^{a,b}$	0.600	0.013	*
17:0 isobr	0.073	0.002	0.066	0.010	0.077	0.005	0.497	NS
17:0	0.327	0.015	0.298	0.022	0.291	0.014	0.220	NS
16:3*n*-4	0.534	0.026	0.601	0.030	0.529	0.033	0.189	NS
18:0	1.47	0.073	1.27	0.076	1.37	0.055	0.205	NS
18:1*n*-9	42.1	0.732	38.7	1.18	41.1	1.07	0.082	NS
18:1*n*-7	1.57	0.160	1.57	0.139	1.40	0.164	0.690	NS
18:2*n*-6	21.0	0.359	20.1	0.731	21.5	0.457	0.282	NS
19:0	0.074	0.022	0.058	0.016	0.084	0.009	0.376	NS
18:3*n*-4	0.208	0.011	0.197	0.017	0.206	0.012	0.863	NS
18:3*n*-3	1.89^{a}	0.057	1.48^{b}	0.054	1.42^{b}	0.031	<0.001	***
18:4*n*-3	0.106	0.013	0.080	0.013	0.120	0.013	0.104	NS
20:1*n*-9	0.198^{b}	0.022	0.271^{a}	0.023	0.276^{a}	0.025	0.042	*
20:2*n*-6	ND	-	ND	-	ND	-	-	-
20:4*n*-6	ND	-	ND	-	ND	-	-	-
20:5*n*-3	ND	-	ND	-	ND	-	-	-
22:6*n*-3	ND	-	ND	-	ND	-	-	-
Partial sums and ratios								
Total SFA	22.5	0.708	23.6	1.01	22.2	0.922	0.578	NS
Total MUFA	53.8	0.538	53.9	0.412	54.0	0.713	0.956	NS
Total PUFA	23.7	0.372	22.5	0.763	23.8	0.456	0.305	NS
Total *n*-3	2.00^{a}	0.048	1.57^{b}	0.058	1.55^{b}	0.030	<0.001	***
Total *n*-6	21.0	0.359	20.1	0.731	21.5	0.457	0.282	NS
SCD	2.66	0.096	2.58	0.111	2.70	0.128	0.750	NS
n-3/*n*-6	0.095^{a}	0.003	0.079^{b}	0.004	0.072^{b}	0.002	<0.001	***

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group. *n*=8 *per* group. ND, not detected. Total SFA = 11:0 + 15:0 + 15:0 + 16:0 + 17:0 isobr + 17:0 + 18:0 + 19:0; Total MUFA = 16:1*n*-9 + 16:1*n*-7 + 18:1*n*-9 + 18:1*n*-7 + 20:1*n*-9; Total PUFA = 16:3*n*-4 + 18:2*n*-6 + 18:3*n*-4 + 18:3*n*-3 + 18:4*n*-3 + 20:2*n*-6 + 20:4*n*-6 + 22:6*n*-3; Total *n*-3 = 18:3*n*-3 + 18:4*n*-3 + 22:6*n*-3; Total *n*-6 = 18:2*n*-6 + 20:2*n*-6 + 20:4*n*-6; SCD ratio = (18:1*n*-7 + 18:1*n*-9 + 16:1*n*-7)/(16:0 + 18:0). Values are means ± SE. Statistical significance: NS = not significantly different, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [a,b,c] Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $P<0.05$).

Even if total MUFA was kept similar across dietary groups ($P>0.05$), 20:1*n*-9 was lower in the LSO group in comparison to the others ($P<0.01$). Total PUFA, as well as the *n*-6 family, showed no variations across dietary groups ($P>0.05$), including LA ($P>0.05$) and AA ($P>0.05$). In contrast, the LSO group had the highest percentage of *n*-3 PUFA and *n*-3/*n*-6 ratio in comparison to FO and FO-EE groups ($P<0.001$). Contrarily to subcutaneous and visceral WAT, DHA was found in interscapular BAT, but not EPA. SCD ratio was kept unchanged across diets ($P>0.05$).

Table 8. Fatty acid composition (mol% of total fatty acids) of visceral white adipose tissue.

	LSO		FO		FO-EE		*P* value	Significance
	Mean	SE	Mean	SE	Mean	SE		
11:0	0.115	0.008	0.114	0.011	0.120	0.007	0.839	NS
14:0	1.82	0.109	1.95	0.157	2.02	0.075	0.341	NS
15:0	0.693	0.031	0.683	0.054	0.690	0.032	0.986	NS
16:0	20.9	0.675	22.1	0.764	22.5	0.386	0.157	NS
16:1*n*-9	0.683[a]	0.023	0.559[b]	0.052	0.596[b]	0.025	0.024	*
16:1*n*-7	8.65[b]	0.347	12.1[a]	0.978	10.6[a]	0.311	<0.001	***
17:0 isobr	0.104	0.004	0.093	0.008	0.105	0.003	0.334	NS
17:0	0.405	0.016	0.389	0.046	0.364	0.019	0.291	NS
16:3*n*-4	0.552	0.024	0.613	0.035	0.542	0.031	0.272	NS
18:0	1.76	0.064	1.53	0.124	1.57	0.047	0.070	NS
18:1*n*-9	40.9[a]	0.752	37.5[b]	0.813	38.2[b]	0.568	0.011	*
18:1*n*-7	1.08	0.126	1.42	0.140	1.20	0.176	0.234	NS
18:2*n*-6	19.8	0.266	18.9	0.602	19.5	0.218	0.443	NS
19:0	0.106	0.009	0.086	0.008	0.082	0.004	0.069	NS
18:3*n*-4	0.220[a]	0.013	0.170[b]	0.008	0.166[b]	0.009	0.006	**
18:3*n*-3	1.97[a]	0.065	1.48[b]	0.049	1.36[b]	0.042	<0.001	***
18:4*n*-3	0.098	0.010	0.087	0.011	0.110	0.003	0.119	NS
20:1*n*-9	0.176[b]	0.011	0.292[a]	0.031	0.263[a]	0.009	<0.001	***
20:2*n*-6	ND	-	ND	-	ND	-	-	-
20:4*n*-6	ND	-	ND	-	ND	-	-	-
20:5*n*-3	ND	-	ND	-	ND	-	-	-
22:6*n*-3	ND	-	ND	-	ND	-	-	-
Partial sums and ratios								
Total SFA	25.9	0.778	26.9	0.949	27.4	0.508	0.285	NS
Total MUFA	51.5	0.520	51.8	0.561	50.9	0.548	0.504	NS
Total PUFA	22.6	0.332	21.3	0.621	21.7	0.221	0.065	NS
Total *n*-3	2.06[a]	0.066	1.57[b]	0.050	1.47[b]	0.040	<0.001	***
Total *n*-6	19.8	0.266	18.9	0.602	19.5	0.218	0.443	NS
SCD	2.25	0.085	2.18	0.098	2.09	0.056	0.274	NS
n-3/*n*-6	0.104[a]	0.003	0.083[b]	0.003	0.075[b]	0.002	<0.001	***

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group. *n*=8 *per* group. ND, not detected. Total SFA = 11:0 + 15:0 + 15:0 + 16:0 + 17:0 isobr + 17:0 + 18:0 + 19:0; Total MUFA = 16:1*n*-9 + 16:1*n*-7 + 18:1*n*-9 + 18:1*n*-7 + 20:1*n*-9; Total PUFA = 16:3*n*-4 + 18:2*n*-6 + 18:3*n*-4 + 18:3*n*-3 + 18:4*n*-3 + 20:2*n*-6 + 20:4*n*-6 + 22:6*n*-3; Total *n*-3 = 18:3*n*-3 + 18:4*n*-3 + 22:6*n*-3; Total *n*-6 = 18:2*n*-6 + 20:2*n*-6 + 20:4*n*-6; SCD ratio = (18:1*n*-7 + 18:1*n*-9 + 16:1*n*-7)/(16:0 + 18:0). Values are means ± SE. Statistical significance: NS = not significantly different, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [a,b,c] Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $P<0.05$).

4.6 Effect of fat depot on gene expression levels of aquaporins and lipid sensitive mediators

The effect of fat depot (sWAT, vWAT and BAT) on the relative expression levels of *AQP3*, *AQP5* and *AQP7*, as well as lipid sensitive mediators (*ADIPO*, *LEP*, *GLUT4*, *PPARγ* and *PPARα*) *per* dietary treatment, LSO, FO and FO-EE is illustrated in Figures 9, 10 and 11.

Table 9. Fatty acid composition (mol% of total fatty acids) of interscapular brown adipose tissue.

	LSO		FO		FO-EE		*P* value	Significance
	Mean	SE	Mean	SE	Mean	SE		
11:0	0.076	0.007	0.074	0.008	0.078	0.003	0.870	NS
14:0	1.32	0.089	1.59	0.075	1.44	0.030	0.074	NS
15:0	0.386	0.032	0.356	0.021	0.353	0.010	0.609	NS
16:0	19.4[b]	0.491	21.2[a]	0.550	20.7[a]	0.332	0.039	*
16:1*n*-9	0.591	0.014	0.534	0.033	0.569	0.017	0.250	NS
16:1*n*-7	5.69	0.436	7.40	0.864	6.03	0.396	0.231	NS
17:0 isobr	0.096	0.007	0.098	0.006	0.101	0.005	0.815	NS
17:0	0.449	0.017	0.440	0.040	0.461	0.023	0.881	NS
16:3*n*-4	0.420	0.032	0.471	0.014	0.433	0.024	0.204	NS
18:0	4.546	0.333	4.73	0.264	4.82	0.242	0.806	NS
18:1*n*-9	41.4	0.717	39.5	0.689	39.9	0.600	0.152	NS
18:1*n*-7	1.08	0.047	1.15	0.070	1.14	0.091	0.656	NS
18:2*n*-6	21.4	0.414	19.7	0.789	21.0	0.372	0.193	NS
19:0	0.155	0.012	0.129	0.009	0.131	0.017	0.243	NS
18:3*n*-4	0.230	0.010	0.208	0.013	0.218	0.009	0.412	NS
18:3*n*-3	1.96[a]	0.057	1.37[b]	0.029	1.30[b]	0.033	<0.001	***
18:4*n*-3	0.080	0.010	0.057	0.009	0.072	0.012	0.224	NS
20:1*n*-9	0.293[b]	0.028	0.424[a]	0.029	0.402[a]	0.018	0.005	**
20:2*n*-6	0.111	0.014	0.121	0.011	0.134	0.008	0.060	NS
20:4*n*-6	0.451	0.062	0.374	0.023	0.434	0.023	0.167	NS
20:5*n*-3	ND	-	ND	-	ND	-	-	-
22:6*n*-3	ND	-	0.242	0.032	0.236	0.013	0.873	NS
Partial sums and ratios								
Total SFA	26.3[b]	0.663	28.6[a]	0.445	28.1[a]	0.400	0.031	*
Total MUFA	49.0	0.727	49.0	0.802	48.1	0.681	0.567	NS
Total PUFA	24.6	0.444	22.4	0.846	23.8	0.399	0.083	NS
Total *n*-3	2.02[a]	0.053	1.56[b]	0.067	1.58[b]	0.043	<0.001	***
Total *n*-6	21.9	0.438	20.2	0.799	21.6	0.396	0.178	NS
SCD	2.03	0.078	1.86	0.045	1.85	0.050	0.141	NS
n-3/*n*-6	0.092[a]	0.002	0.078[b]	0.003	0.073[b]	0.002	<0.001	***

LSO, the linseed oil group (the control group); FO, the fish oil group; FO-EE, the fish oil ethyl esters group. *n*=8 *per* group. ND, not detected. Total SFA = 11:0 + 15:0 + 15:0 + 16:0 + 17:0 isobr + 17:0 + 18:0 + 19:0; Total MUFA = 16:1*n*-9 + 16:1*n*-7 + 18:1*n*-9 + 18:1*n*-7 + 20:1*n*-9; Total PUFA = 16:3*n*-4 + 18:2*n*-6 + 18:3*n*-4 + 18:3*n*-3 + 18:4*n*-3 + 20:2*n*-6 + 20:4*n*-6 + 22:6*n*-3; Total *n*-3 = 18:3*n*-3 + 18:4*n*-3 + 22:6*n*-3; Total *n*-6 = 18:2*n*-6 + 20:2*n*-6 + 20:4*n*-6; SCD ratio = (18:1*n*-7 + 18:1*n*-9 + 16:1*n*-7)/(16:0 + 18:0). Values are means ± SE. Statistical significance: NS = not significantly different, $P>0.05$; *, $P<0.05$; **, $P<0.01$; ***, $P<0.001$. [a,b,c] Means in the same row with different superscripts are statistically different (Tukey's *post hoc*, $P<0.05$).

Except for *AQP7*, a clear effect of fat depot was observed for *AQP3*, *AQP5* and lipid sensitive mediators ($P<0.05$). This effect is most certainly associated with the lower transcriptional profile level of *AQP3*, *ADIPO*, *LEP*, *GLUT4* and *PPARγ* in BAT. LEP is almost exclusively produced by white adipocytes in proportion to their TAG storage. ADIPO and TAG accumulation are also strictly associated and may be foreseen as indicators of adequate differentiation and proper functioning of adipocyte cells. Conversely, *PPARα* is

highly expressed in BAT in comparison with residual levels of mRNA expression found in subcutaneous and visceral WAT depots ($P<0.05$), which certainly relates to fatty acid β-oxidation occurring in brown adipocytes, as a thermogenic organ. The same trend applies to *AQP5* ($P<0.001$), a water channel protein. The variation pattern between subcutaneous and visceral WAT is similar for some genes, including *AQP3, AQP5, AQP7, LEP* and *GLUT4* ($P>0.05$).

In both LSO (Figure 9A) and FO (Figure 9B) dietary treatments non-variations occurred across fat depots, although in FO-EE (Figure 9C) it is clear that *AQP5* mRNA levels in BAT were much higher than in the two WAT. For *AQP7*, no variations were obtained in the three fat depots, regardless the dietary group ($P>0.05$) (Figure 9). *GLUT4* was found down-regulated in brown fat in comparison to white ($P<0.05$).

4.7 Effect of dietary treatment on gene expression levels of aquaporins and lipid sensitive mediators

Figure 12 illustrates the variations of *AQP3, AQP5* and *AQP7 per* adipose tissue, white subcutaneous (panel A), white visceral (panel B) and brown (panel C), according to LSO, FO and FO-EE diets. The main finding is that the thermogenic BAT is more sensitive to *n*-3 PUFA molecular structures than both WAT depots.

In fact, all three AQPs (*AQP3, AQP5* and *AQP7*) did not vary according to dietary treatment in subcutaneous and visceral WAT ($P>0.05$). An inverse pattern of variation was found for *AQP3* and *AQP5* ($P<0.05$) between FO and FO-EE diets ($P<0.05$) in BAT suggesting that these AQPs might act as complementary.

The variations of mRNA expression levels of adipokines, *ADIPO* and *LEP* per adipose tissue, white subcutaneous (panel A) white visceral (panel B) and brown (panel C), according to LSO, FO and FO-EE diets are presented in Figure 13. In sWAT, the relative levels of *ADIPO* were higher in FO group comparing to FO-EE ($P<0.05$) (Figure 13A). The inverse occurred in vWAT with higher *ADIPO* expression in FO-EE relative to FO ($P<0.01$) (Figure 13B). With a 10-fold lower expression in relation to both WAT, the gene expression of *ADIPO* in BAT was up-regulated in FO diet compared to the other dietary treatments ($P<0.05$) (Figure 13C). In what *LEP* concerns, only BAT was affected by diet with higher mRNA levels in the LSO group compared to FO-EE ($P<0.05$) (Figure 13C).

The gene expression levels of the remaining lipid sensitive mediators, *GLUT4*, *PPARα* and *PPARγ* controlling fat metabolism *per* adipose tissue (subcutaneous WAT, visceral WAT and BAT), according to LSO, FO and FO-EE diets are presented in Figure 14.

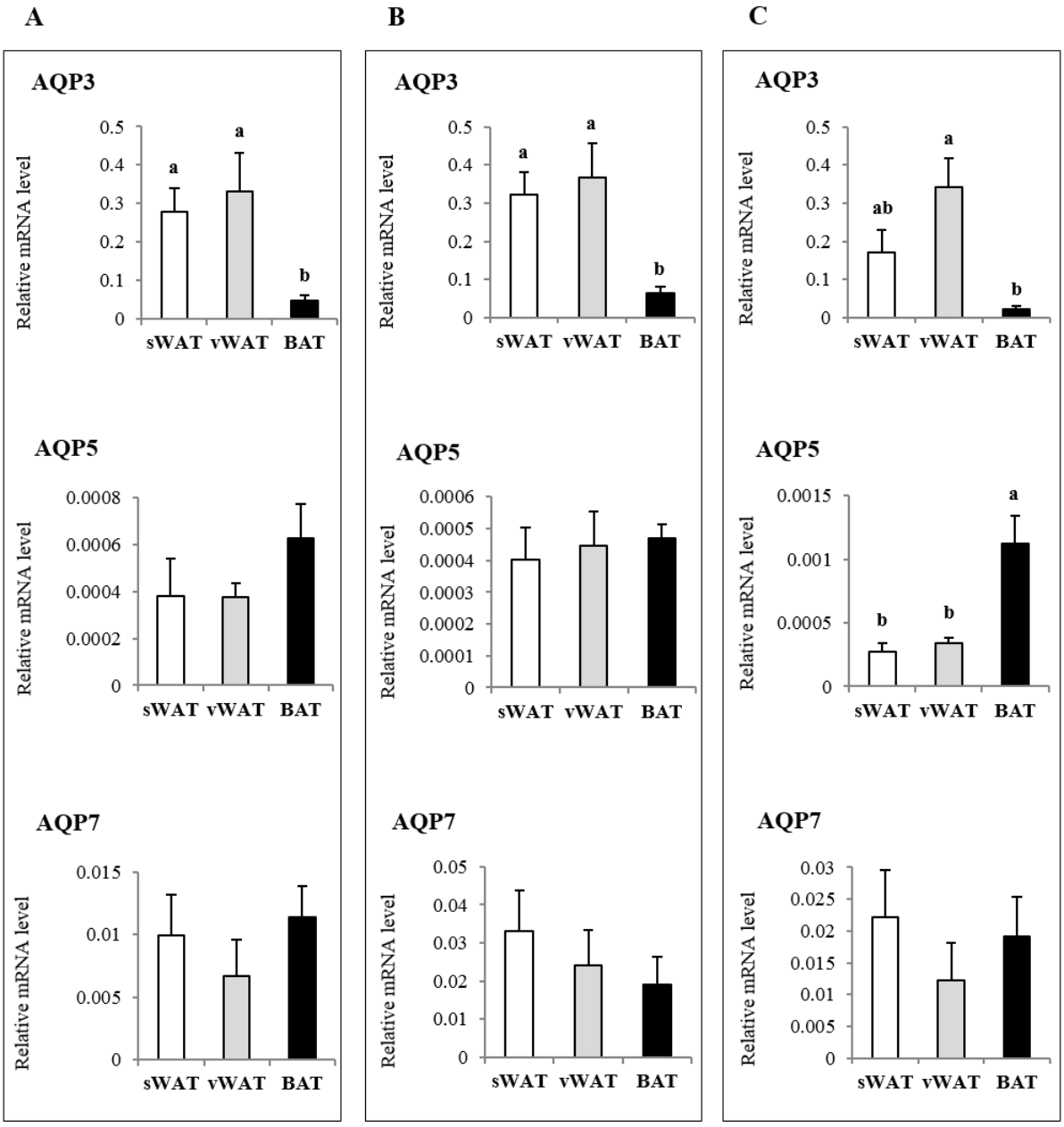

Figure 9. Effect of fat depot on the relative expression levels of aquaporin-3 (*AQP3*), aquaporin-5 (*AQP5*) and aquaporin-7 (*AQP7*) *per* dietary treatment, LSO (A), FO (B) and FO-EE (C) from hamsters. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

Concerning the mRNA levels of *GLUT4* and *PPARγ*, the pattern and the values were similar in sWAT, with an up-regulation occurring in hamsters fed on FO relative to FO-EE ($P<0.05$, $P<0.01$, respectively) (Figure 14A). The gene expression levels of *PPARγ* in BAT were equally higher in FO and FO-EE comparing to the control ($P<0.01$) (Figure 14C). No changes in *PPARα* expression levels were obtained across dietary groups *per* fat depot ($P>0.05$).

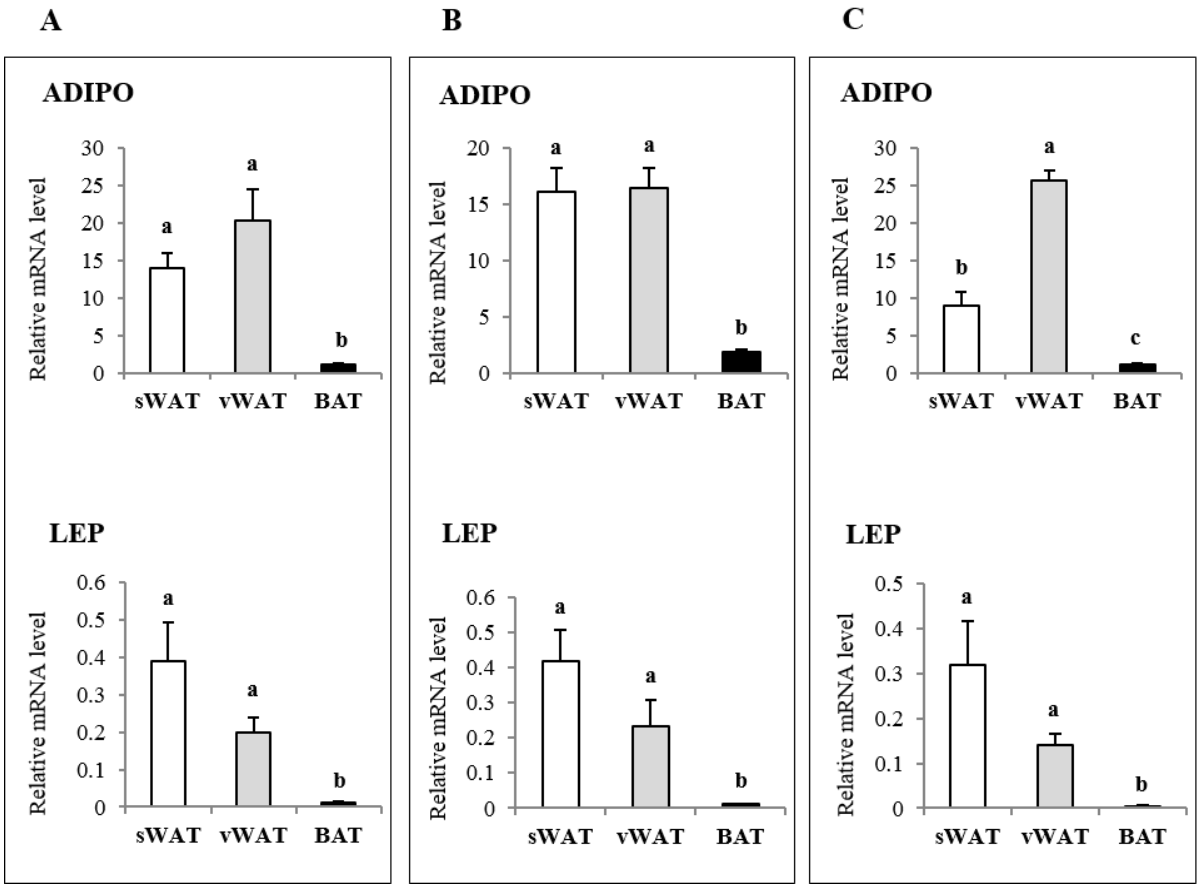

Figure 10. Effect of fat depot on the relative expression levels of adipokines, adiponectin (*ADIPO*) and leptin (*LEP*) *per* dietary treatment, LSO (A), FO (B) and FO-EE (C) from hamsters. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

In vWAT and BAT, no variations in *GLUT4* mRNA levels were observed ($P>0.05$) (Figures 14B, 14C). The same occurred with PPARγ in vWAT ($P>0.05$) (Figure 14B).

Once again, BAT appears more sensitive to *n*-3 PUFA diets, followed by subcutaneous and visceral WAT, in this specific order. The molecular structure of combined EPA and DHA as EE reduced the majority of mRNA levels. It is the case of *ADIPO*, *GLUT4* and *PPARγ* in subcutaneous WAT ($P<0.05$) as well as *ADIPO* and *LEP* in BAT ($P<0.05$).

The only exception that is worth noticing is *ADIPO*, whose mRNA levels were found increased in the FO-EE diet relative to FO in visceral WAT ($P<0.05$). The other lipid sensitive mediators remained unchanged in visceral WAT ($P>0.05$).

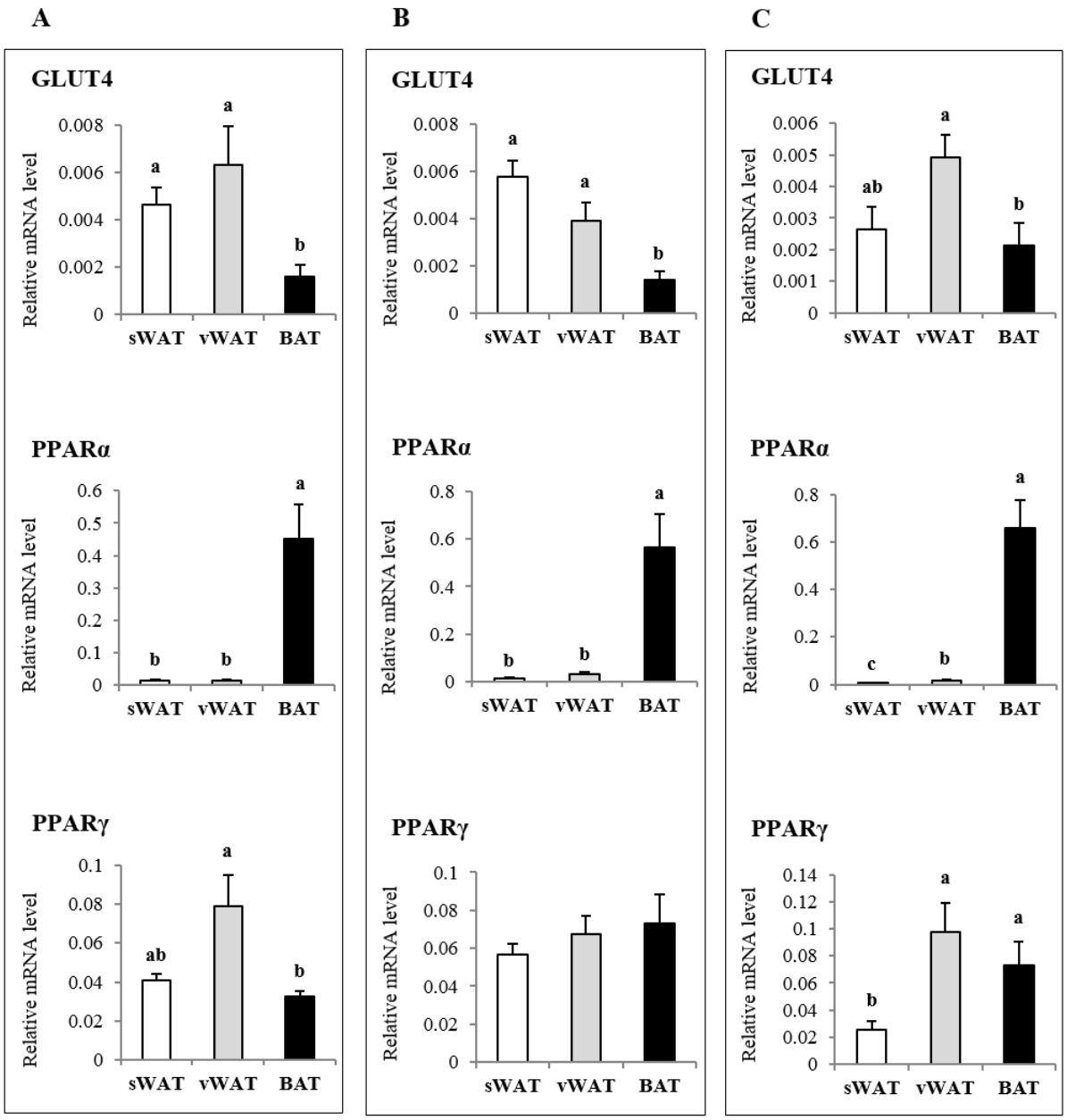

Figure 11. Effect of fat depot on the relative expression levels of glucose transporter type 4, (*GLUT4*), peroxisome proliferator activator receptor alpha (*PPARα*) and peroxisome proliferator activator receptor gamma (*PPARγ*) *per* dietary treatment, LSO (A), FO (B) and FO-EE (C) from hamsters. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

4.8 Interaction effect of fat depot × diet on gene expression levels of aquaporins and lipid sensitive mediators

At last, Figures 15, 16 and 17 integrate in the same statistical model the effects of fat depot (subcutaneous WAT, visceral WAT and BAT), diet (LSO, FO, FO-EE) and diet × fat depot interaction on gene expression levels of aquaporins and lipid sensitive mediators.

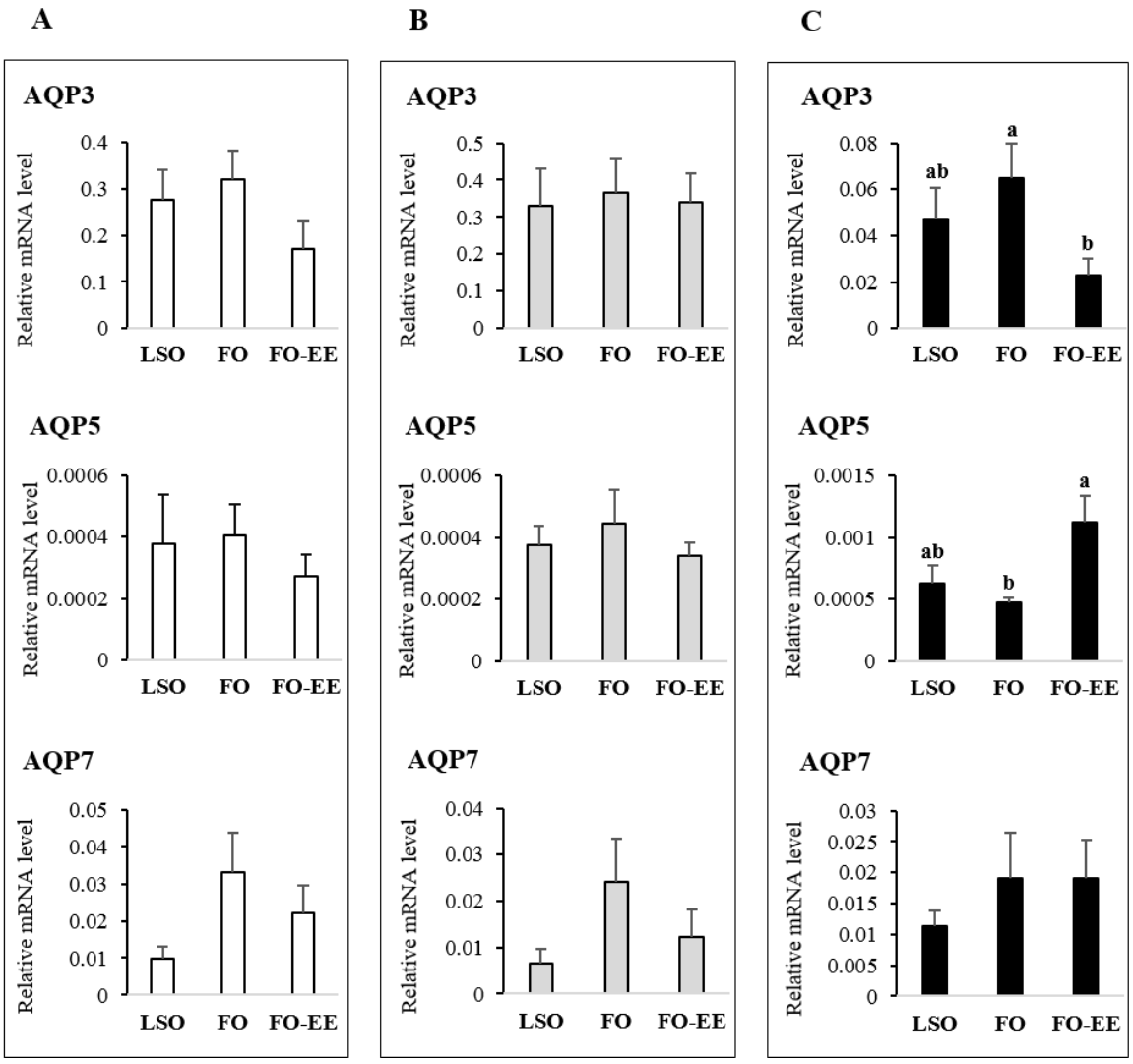

Figure 12. Effect of dietary treatments on the relative expression levels of aquaporin-3 (*AQP3*), aquaporin-5 (*AQP5*) and aquaporin-7 (*AQP7*) in the subcutaneous white adipose tissue (A), in the visceral white adipose tissue (B) and in the interscapular brown adipose tissue (C) from hamsters fed on LSO, FO and FO-EE diets. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

Most of the variations found regarding fat depot and diet effects reflect the ones already described above and represented from Figure 1 to Figure 14.

In *AQP3* expression, it was found a clear effect of fat depot ($P<0.001$), meaning that regardless the dietary treatment the values of expression of this gene were distinct among adipose tissues. In this case, both WAT had higher *AQP3* mRNA levels comparing to BAT ($P<0.001$). An interaction between diet and fat depot ($P=0.031$) was found for the expression level of *AQP5*, and also a fat depot effect ($P=0.001$). The reason for this is that BAT presented higher mRNA levels of *AQP5* than subcutaneous and visceral WAT for the FO-EE diet. *AQP7* was only affected by

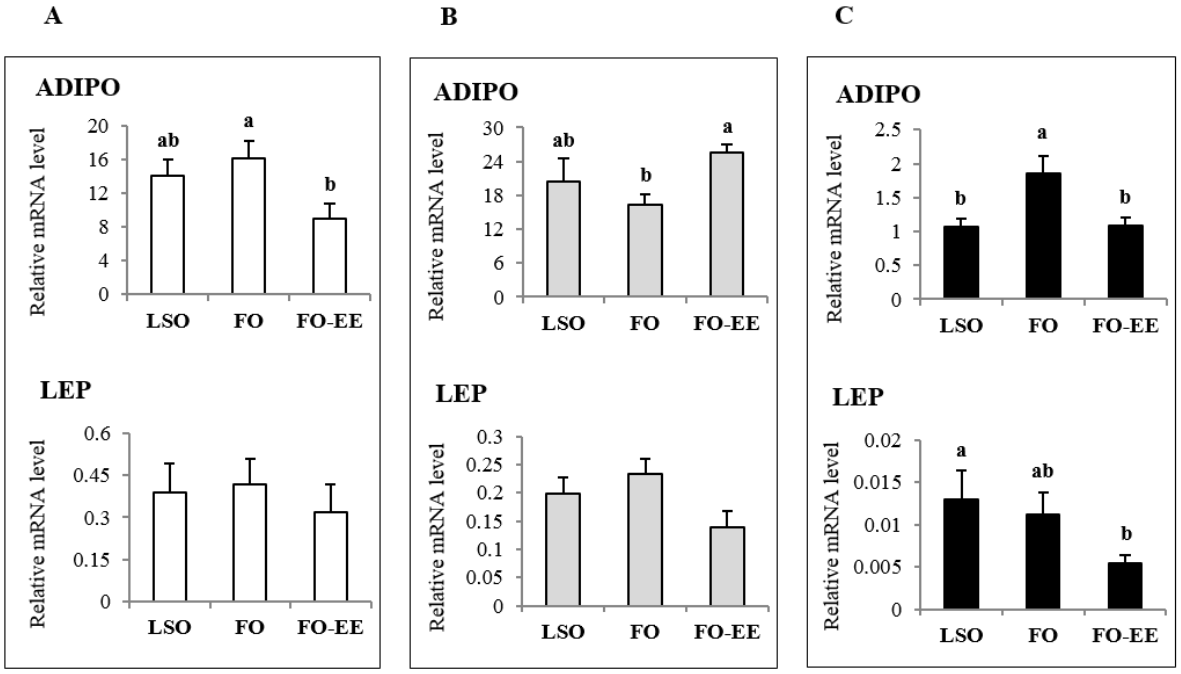

Figure 13. Effect of dietary treatments on the relative expression levels of adipokines, adiponectin (*ADIPO*) and leptin (*LEP*) in the subcutaneous white adipose tissue (A), in the visceral white adipose tissue (B) and in the interscapular brown adipose tissue (C) from hamsters fed on LSO, FO and FO-EE diets. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

diet ($P=0.005$) with higher levels of mRNA found in the FO dietary treatment in comparison to LSO and FO-EE suggesting a beneficial effect of EPA and DHA combined as TAG (Figure 15).

For *ADIPO* gene, a clear effect of fat depot was depicted ($P<0.001$) being the mRNA levels of this adipokine lower in BAT than in sWAT and vWAT. It was also observed an interaction between diet and fat depot justified by the occurrence of higher mRNA levels in FO diet relative to FO-EE in sWAT and BAT, but the inverse was found for vWAT ($P<0.001$). The *LEP* gene exhibited also a fat depot effect ($P<0.001$) with residual levels of mRNA found in BAT in comparison to both WAT (Figure 16).

In *GLUT4*, it was observed a fat depot effect ($P<0.001$) with BAT having once again lower expression levels than sWAT and vWAT. In addition, *GLUT4* was affected by an interaction between diet and fat depot ($P=0.022$) which is explained by decreased mRNA levels in the FO-EE diet in subcutaneous WAT, not verified in BAT and visceral WAT. A fat depot effect ($P<0.001$) was observed for *PPARα* gene with BAT having much higher expression levels than subcutaneous and visceral WAT for which the mRNA levels found were almost undetected.

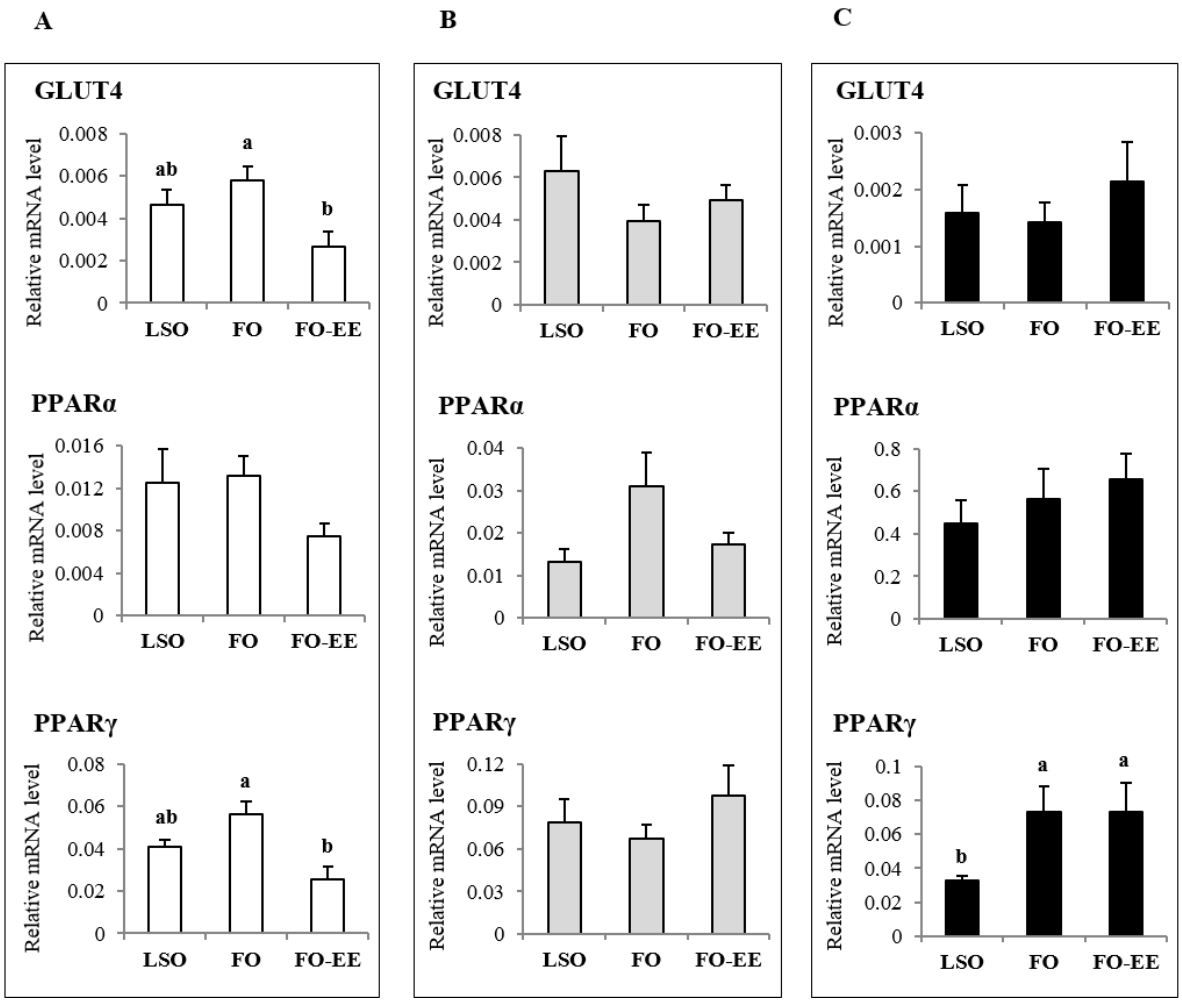

Figure 14. Effect of dietary treatments on the relative expression levels of glucose transporter type 4 (*GLUT4*), peroxisome proliferator activated receptor alpha (*PPARα*) and peroxisome proliferator activated receptor gamma (*PPARγ*) in the subcutaneous white adipose tissue (A), in the visceral white adipose tissue (B) and in the interscapular brown adipose tissue (C) from hamsters fed on LSO, FO and FO-EE diets. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

For *PPARγ*, a fat depot effect ($P<0.001$) was found with visceral WAT exhibiting higher expression levels relative to subcutaneous WAT and BAT. Still for *PPARγ*, FO-EE fed hamsters had lower mRNA levels than FO in subcutaneous WAT but identical in visceral WAT and BAT justifying the interaction detected between diet and fat depot ($P=0.004$) (Figure 17).

In summary, the lowest values of mRNA levels were consistently found for *AQP3*, *ADIPO*, *LEP* and *GLUT4* in BAT relative to both WAT, and the opposite was clearly observed for *PPARα* (Figures 15, 16 and 17).

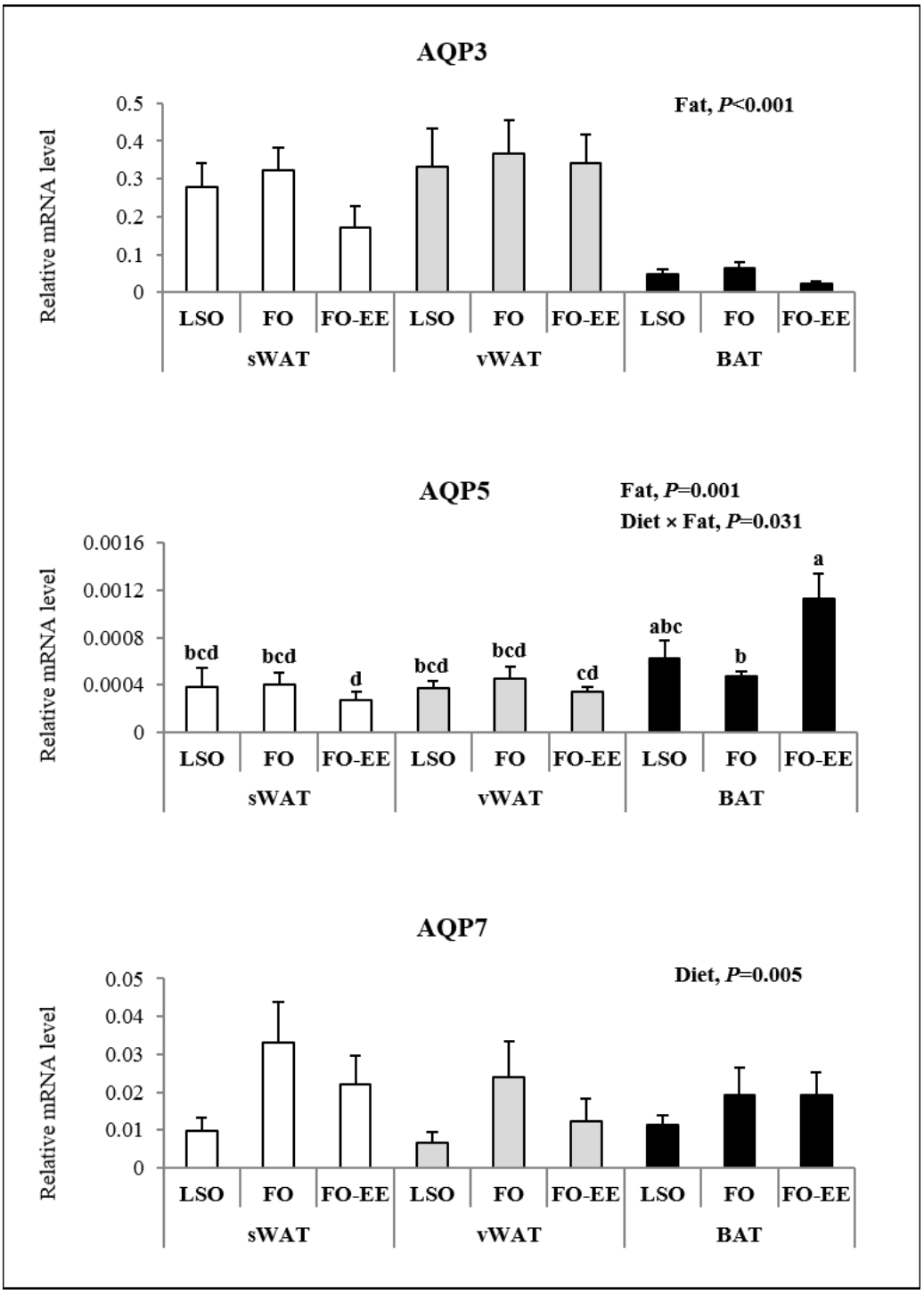

Figure 15. Effect of fat depot, diet and diet × fat depot interaction on the relative expression levels of aquaporin-3 (*AQP3*), aquaporin-5 (*AQP5*) and aquaporin-7 (*AQP7*) in the subcutaneous white adipose tissue (sWAT, white bars), visceral white adipose tissue (vWAT, gray bars) and brown adipose tissue (BAT, black bars) from hamsters fed on LSO, FO and FO-EE. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

The statistical tool of interaction (diet × fat depot) allowed us to validate our initial hypothesis that different patterns of aquaporins and lipid sensitive mediators expression exist between WAT (subcutaneous and visceral) and BAT when hamsters are fed on specific *n*-3

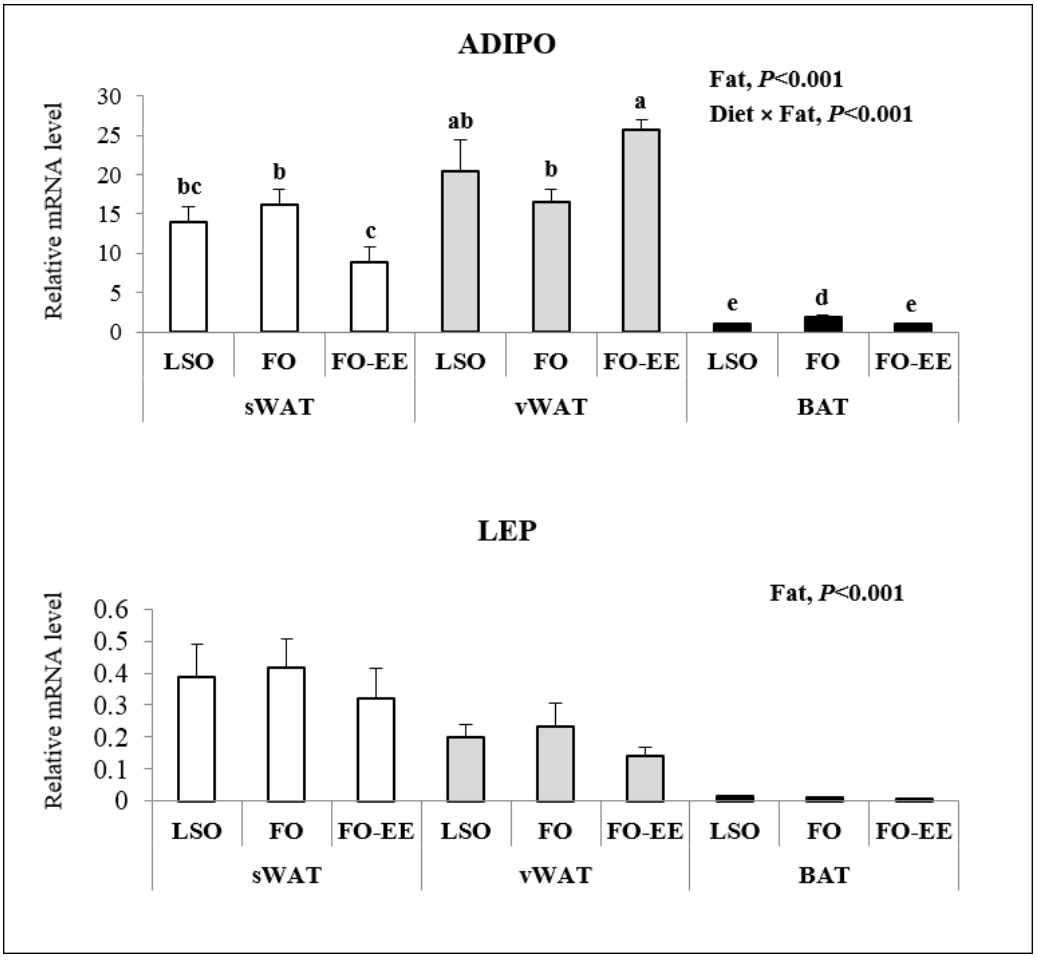

Figure 16. Effect of fat depot, diet and diet × fat depot interaction on the relative expression levels of adiponectin (*ADIPO*) and leptin (*LEP*) in the subcutaneous white adipose tissue (sWAT, white bars), visceral white adipose tissue (vWAT, gray bars) and brown adipose tissue (BAT, black bars) from hamsters fed on LSO, FO and FO-EE. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

PUFA molecular structures: FO (rich in EPA and DHA in the TAG form) and FO-EE (rich in EPA and DHA in the EE form) *versus* LSO (rich in ALA, 18:3*n*-3, taken as the reference group).

4.9 Correlation between fatty acids and gene expression levels *per* fat depot

Table 10 presents the Pearson's correlation coefficients (*r*) for fatty acids, partial sums of fatty acids and ratios, and gene expression levels in sWAT (A), vWAT (B) and BAT (C).

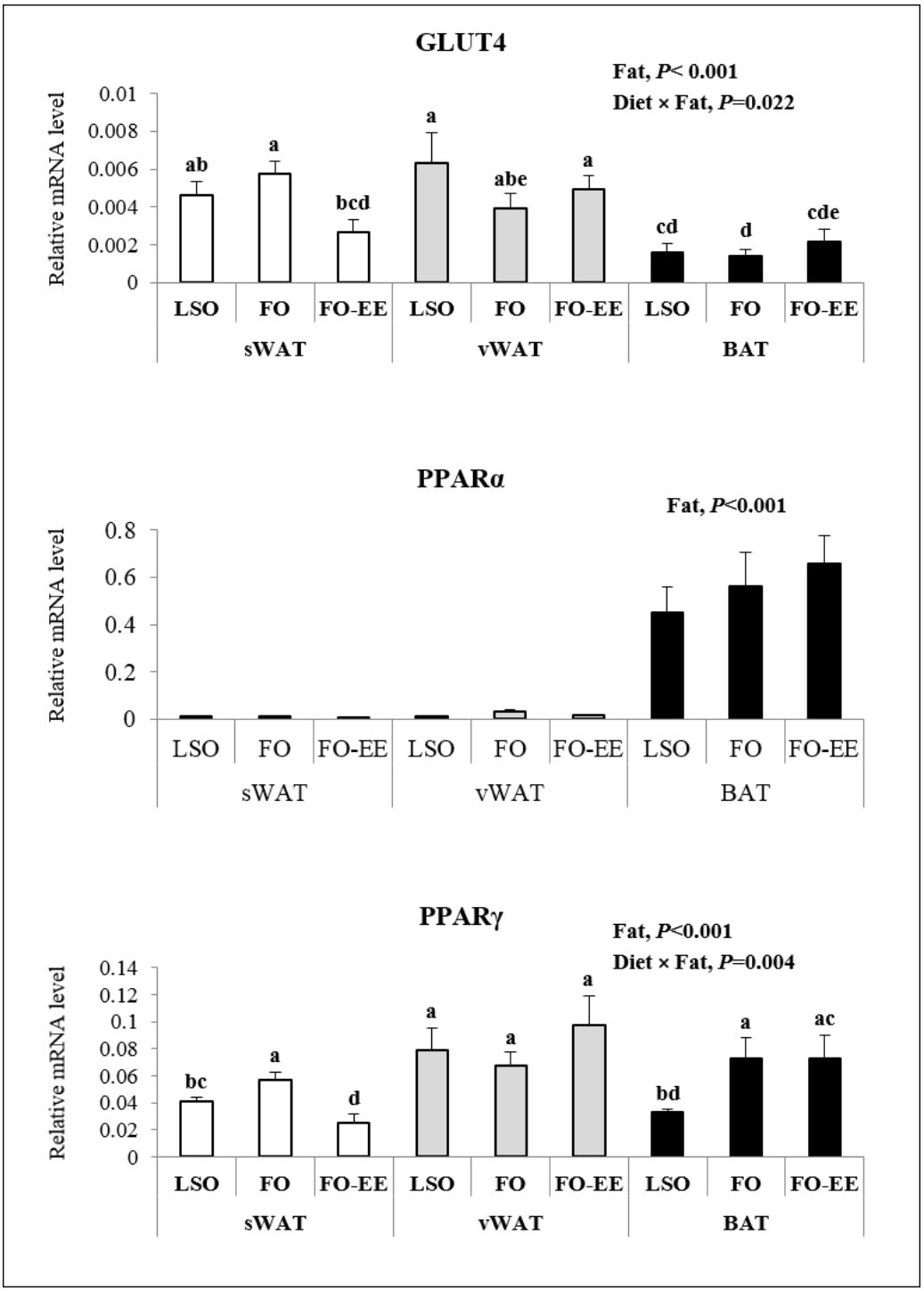

Figure 17. Effect of fat depot, diet and diet × fat depot interaction on the relative expression levels of glucose transporter type 4 (*GLUT4*), peroxisome proliferator receptor alpha (*PPARα*) and peroxisome proliferator receptor gamma (*PPARγ*) in the subcutaneous white adipose tissue (sWAT, white bars), visceral white adipose tissue (vWAT, gray bars) and brown adipose tissue (BAT, black bars) from hamsters fed on LSO, FO and FO-EE. Means ± standard error (SE). Means with different letters are significantly different (Tukey's *post hoc*, $P<0.05$).

In sWAT, *AQP3* was positively correlated with *ADIPO* ($P<0.01$) and *AQP7* ($P<0.01$). *ADIPO* was positively correlated with *PPARγ* ($P<0.01$), *PPARα* ($P<0.01$), and *LEP* ($P<0.01$). A positive correlation was observed between *GLUT4* and *PPARγ* ($P<0.001$), *GLUT4* and *PPARα* ($P<0.05$), and between *PPARα* and *PPARγ* ($P<0.01$) (Table 10A).

In vWAT, *AQP3* was positively correlated with *AQP7* ($P<0.05$) and *AQP5* ($P<0.05$). *PPARα* was negatively correlated with total PUFA ($P<0.01$) and *n*-6 PUFA ($P<0.01$). Moreover, a negative correlation was also found between PPARγ and total MUFA ($P<0.05$) (Table 10B).

In BAT, *AQP7* correlated positively with 22:6*n*-3 fatty acid ($P<0.05$), *PPARγ* ($P<0.05$) and *LEP* ($P<0.05$). *ADIPO* was correlated with both *PPARγ* ($P<0.01$) and *LEP* ($P<0.05$). *PPARα* correlated with both *GLUT4* ($P<0.05$) and *PPARγ* ($P<0.01$) (Table 10C). It is worth notice that all correlations found, either positive or negative, were moderate ($0.7 \geq r \geq 0.3$) (Tables 10A, 10B and 10C).

4.10 PCA on adipose tissues' fatty acids and gene expression levels

Figure 18A displays the projection of the first (PC1) and second (PC2) principal components in the plane using the gene expression levels of aquaporins (*AQP3*, *AQP5* and *AQP7*) and lipid sensitive mediators (*ADIPO*, *LEP*, *GLUT4*, *PPARα* and *PPARγ*) and the percentage of fatty acids common to all three fat depots (sWAT, vWAT and BAT). Both PC combined explained 32.89% of the total variance. The PC1 was characterized by variables with positive loadings, such as 14:0 (0.85), 16:1*n*-7 (0.81), 15:0 (0.75), 16:0 (0.73), and 16:3*n*-4 (0.55), and by variables with negative loadings, 18:1*n*-9 (-0.78), 18:2*n*-6 (-0.73), 18:3*n*-4 (-0.70) and 18:0 (-0.58) (Figure 18A). The PC2 was positively defined by 18:1*n*-7 (0.72), and negatively by 17:0 (-0.75) and 16:1*n*-9 (-0.59) (Figure 18A). All genes were unrelated to PC1 and PC2. *AQP3*, *AQP5* and *AQP7* were clustered in quadrant (b) of Figure 18A. *ADIPO* and *LEP* were positioned in the right side of the plane (Figure 18A). *n*-3 PUFA, 18:3*n*-3 and 18:4*n*-3 appeared very close to each other in quadrant (d) of Figure 18A. SFA were dispersed across quadrants (a), (c) and (d) while MUFA were located in quadrants (a), (b) and (c) of Figure 18A.

The projection of scores in the PC1 × PC2 plane set apart three clusters matching the three analyzed fat depots: BAT was nearly individualized in quadrant (d), the sWAT was mostly positioned in quadrants (a) and (b), and the vWAT was largely located in quadrant (c) of Figure 18B. The clusters of subcutaneous and visceral WAT showed more dispersion that the one deliminated for BAT (Figure 18B). Thus, the PCA on adipose tissue' fatty acids and

gene expression levels discriminated all fat depots. However, the discrimination of dietary treatments (LSO, FO and FO-EE) within each adipose tissue was unattainable (Figure 18B).

Table 10. Pearson's correlations coefficients among fatty acids, partial sums of fatty acids and ratios, and gene expression levels in subcutaneous white adipose tissue (A), visceral white adipose tissue (B) and brown adipose tissue (C).

A

	20:4*n*-6	**18:3*n*-3**	**22:6*n*-3**	**Total SFA**	**Total MUFA**	**Total PUFA**	***n*-3 PUFA**	***n*-6 PUFA**	***n*-3/*n*-6**	***PPARγ***	***PPARα***	***GLUT4***	***LEP***	***ADIPO***	***AQP7***	***AQP5***
AQP3	-	0.289	-	0.217	-0.255	-0.090	0.219	-0.146	0.282	0.329	0.053	-0.108	0.087	0.570**	0.660**	0.310
AQP5	-	0.078	-	0.189	-0.272	-0.034	0.057	-0.058	0.062	0.036	-0.182	-0.173	-0.018	0.045	0.122	
AQP7	-	-0.188	-	0.220	-0.330	0.026	-0.222	0.040	-0.229	0.335	0.059	0.084	-0.048	0.340		
ADIPO	-	0.193	-	0.308	-0.229	-0.253	0.166	-0.314	0.309	0.630**	0.573**	0.311	0.562**			
LEP	-	0.006	-	0.208	-0.221	-0.107	-0.012	-0.103	0.029	0.269	0.036	0.035				
GLUT4	-	0.151	-	-0.026	-0.078	0.113	0.140	0.089	0.073	0.672***	0.489*					
PPARα	-	0.042	-	0.155	-0.073	-0.165	0.053	-0.192	0.148	0.574**						
PPARγ	-	0.183	-	0.292	-0.292	-0.169	0.150	-0.217	0.243							

B

	20:4*n*-6	**18:3*n*-3**	**22:6*n*-3**	**Total SFA**	**Total MUFA**	**Total PUFA**	***n*-3 PUFA**	***n*-6 PUFA**	***n*-3/*n*-6**	***PPARγ***	***PPARα***	***GLUT4***	***LEP***	***ADIPO***	***AQP7***	***AQP5***
AQP3	-	0.055	-	0.125	-0.285	0.116	0.027	0.094	-0.017	0.072	-0.028	-0.198	-0.166	0.018	0.500*	0.438*
AQP5	-	-0.052	-	0.157	-0.134	-0.114	-0.068	-0.164	-0.034	0.097	0.351	-0.014	0.149	0.199	0.077	
AQP7	-	-0.058	-	-0.026	0.022	0.019	-0.096	0.045	-0.121	0.361	-0.044	-0.089	0.380	-0.024		
ADIPO	-	-0.060	-	-0.127	0.037	0.170	-0.039	0.203	-0.132	0.240	-0.218	-0.197	0.223			
LEP	-	0.107	-	0.089	-0.040	-0.103	0.083	-0.142	0.124	0.119	-0.078	0.015				
GLUT4	-	0.147	-	-0.051	-0.098	0.199	0.166	0.172	0.104	-0.063	-0.053					
PPARα	-	-0.273	-	0.368	-0.041	-0.576**	-0.287	-0.616**	-0.066	-0.049						
PPARγ	-	0.014	-	0.303	-0.444*	0.013	0.004	0.019	-0.009							

C

	20:4*n*-6	**18:3*n*-3**	**22:6*n*-3**	**Total SFA**	**Total MUFA**	**Total PUFA**	***n*-3 PUFA**	***n*-6 PUFA**	***n*-3/*n*-6**	***PPARγ***	***PPARα***	***GLUT4***	***LEP***	***ADIPO***	***AQP7***	***AQP5***
AQP3	-0.174	0.147	0.592	0.189	-0.142	-0.018	0.177	-0.069	0.237	0.012	-0.182	-0.420	-0.194	0.192	0.332	-0.133
AQP5	-0.196	-0.256	-0.219	-0.013	-0.003	0.015	-0.139	0.025	-0.177	-0.151	0.057	0.095	-0.327	-0.386	-0.048	
AQP7	0.360	0.227	0.494*****	0.951	0.989	0.943	0.518	0.906	0.408	0.503*****	0.796	0.667	0.137*****	0.069		
ADIPO	-0.126	-0.114	-0.039	0.218	-0.184	0.002	-0.123	0.008	-0.160	0.574******	0.302	0.015	0.530*****			
LEP	0.220	0.296	0.255	-0.099	-0.041	0.129	0.252	0.101	0.222	0.129	0.244	0.280				
GLUT4	0.358	-0.102	0.100	0.042	-0.277	0.262	-0.030	0.301	-0.206	-0.069	0.500*****					
PPARα	-0.132	-0.252	0.324	0.100	-0.072	-0.013	-0.167	0.012	-0.183	0.594******						
PPARγ	-0.235	-0.356	0.126	0.378	-0.227	-0.093	-0.202	-0.080	-0.185							

*, $P<0.05$; **, $P<0.01$; ***, $P<0.001$.

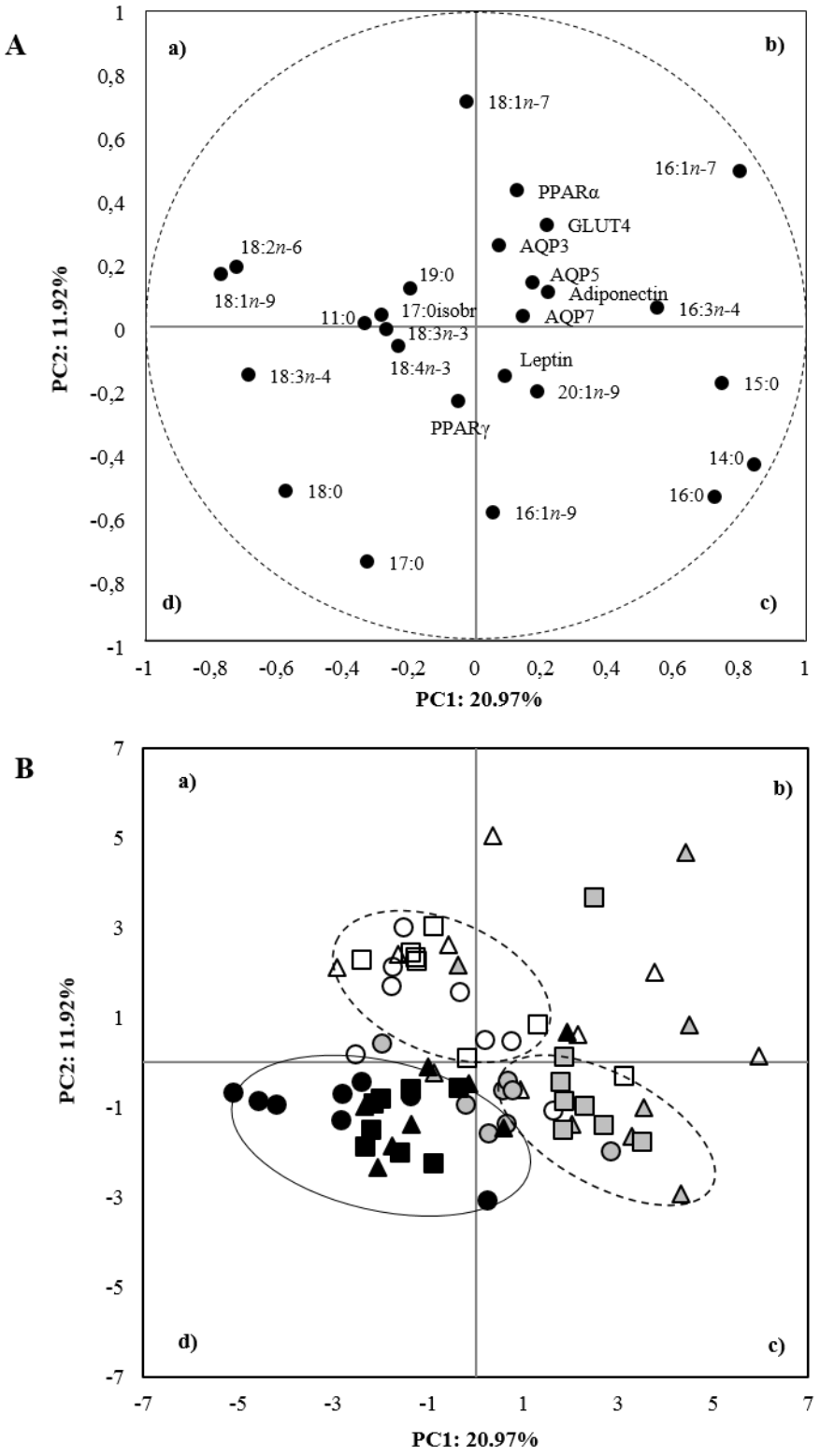

Figure 18. Loadings plot of the first and second principal components (PC) of the pooled FAME and mRNA levels of genes (A) and component's score vectors (B) of sWAT (white markers), vWAT (gray markers) and BAT (black markers) from hamsters fed on LSO (○), FO (Δ) and FO-EE (□) diets.

5. Discussion and conclusion

Over the last decades, many studies established the beneficial effects of diets supplemented with *n*-3 PUFA, notably EPA and DHA, which are believed to have anti-adipogenic properties among their multiple protective actions (Buckley & Howe, 2010).

Since all diets contained the same amount of fat, it was expected that hamsters weight gain and final body weight did not vary among dietary groups, as well as adipose tissue weights from subcutaneous WAT, visceral WAT and BAT. These findings agree with the non-variation of the appetite-suppressing hormone leptin in plasma across *n*-3 PUFA diets (Murphy et al., 2013), and consequently with similar values on hamsters' daily feed intake.

Plasma glucose levels were found decreased in hamsters fed FO and FO-EE diets, which concurs with previous findings (Holness et al., 2003). Actually, EPA and DHA are considered a valuable nutritional tool for preventing insulin resistance in humans associated with obesity. Insulin stimulates fatty acid synthesis in the adipose tissue and liver, as well as formation and storage of TAG in both tissues (Wilcox, 2005). Similar to the results obtained for plasma insulin concentrations, the insulin resistance marker, QUICKI, was identical across dietary treatments, without affecting insulin homeostasis.

Hamsters fed FO-EE diet were the ones with the lowest values of LDL-Cholesterol. However, Mori et al. (2000) and Geppert et al. (2006) studies showed the opposite, stating that highly purified DHA enriched diets increased LDL-Cholesterol (Li et al., 1997; Mori et al., 2000). Also with a positive note is the increment of HDL-Cholesterol in hamsters fed FO diet, even if Ishida et al. (2013) reported a reduction on this parameters in hamsters fed a high-fat diet containing *n*-3 PUFA oils with EPA and DHA (Ishida et al., 2013).

ADIPO and LEP are adipokines responsible for normal adipocyte phenotype and function (Cowherd et al., 1999; Cao, 2014). LEP is almost exclusively produced by adipocytes in proportion to their TAG storage and it is known to regulate lipolysis by controlling the level of hormone sensitive-lipase (Stern et al., 2016). Therefore, the non-variation of systemic adiponectin and leptin agrees with similar fat depots weight found across dietary treatments.

The fatty acid composition of adipose tissue is considered the gold standard for dietary fatty acids (Beynen et al., 1980; Hodson et al., 2008; Abbott et al., 2012). In fact, approximately 99% of fat tissue contains TAG with 0.3% of cholesterol and less than 0.1% of phospholipids (Hirsch et al., 1960). Common to all fat depots (subcutaneous WAT, visceral WAT and BAT), palmitic acid (16:0) was the main SFA while oleic acid (18:1*n*-9) was the

main representative of MUFA. Both 15:0 and 17:0 fatty acids were found in low concentrations, as previously described by other authors (Biong et al., 2006). Oleic and linoleic (18:2*n*-6) acids combined formed the highest proportion of fatty acids. The deposition of 18:2*n*-6 and 18:3*n*-3 fatty acids in TAG adipose tissue consists primarily of those provided by *n*-3 PUFA diets. DHA and EPA were not detected in subcutaneous and visceral WAT**.** The same trend was verified also in BAT for EPA, but not for DHA, reaching the highest percentage of incorporation in FO-EE fed hamsters. As previously reported (Bandarra et al., 2016) the absence of EPA and DHA in white fat depots is due to the preferably incorporation of these *n*-3 PUFA in the brain and liver (Ross et al., 2015). Of note are the very low proportions of very long chain (20 or more carbons) PUFA in subcutaneous WAT, as the fatty acids in this tissue are primarily less than 18 carbons in length.

EPA and DHA display several beneficial effects, such as the ability to lower lipogenesis, increase lipolysis and reduce inflammation, which are advantageous for adipose tissue biology (Todorcevic & Hodson, 2015). Higher proportions of MUFA are found in subcutaneous fat depot when compared to visceral, which appears to be at the expense of SFA. The abundance of EPA and DHA in humans fat does not increase notably even after supplementation. This suggests that EPA and DHA are not preferentially long-term stored in TAG from adipose tissue, rather they may be partitioned to oxidation pathways or storage in other lipid fractions, such as plasma phospholipids, cell phospholipids and red blood cells, all of them having a notably higher abundance of both EPA and DHA than adipose tissue (Lopes et al., 2017).

BAT thermogenesis is influenced by dietary fatty acids, as fatty acids provide the major fuel in brown fat (Williamson, 1970; Mercer & Trayhurn, 1987). *n*-6 and *n*-3 PUFA have been shown to have a stimulatory effect on BAT thermogenesis concomitant with improved insulin sensitivity and glucose metabolism by reducing fat deposition and weight gain in rodents, and preventing obesity (Nedergaard et al., 1983; Cannon & Nedergaard, 2004; Nedergaard & Cannon, 2010).

AQP5 is a selective water channel while AQP3 is an aquaglyceroporin which facilitates permeation of glycerol in addition to water (Madeira et al., 2015b). So, it was expected that *AQP3* would be upregulated in WAT in relation to BAT since white fat is responsible for the deposition of energy in form of TAG. In BAT, the gene expression of *AQP3* decreased when hamsters were fed FO-EE diet, which probably means that the EE molecular form promotes less glycerol flux in brown adipocytes in comparison to FA available as TAG. An inverse pattern of variation was found for *AQP3* and *AQP5* between FO

and FO-EE diets in BAT suggesting that these AQPs might act as complementary. Among the various mammalian aquaglyceroporins, AQP7 is the most representative glycerol channel and was the first to be detected in the adipose tissue (Fruhbeck et al., 2006; da Silva & Soveral, 2017). In the overall picture, *AQP7* was only affected by diet with higher levels of mRNA found in the FO dietary treatment in comparison to LSO and FO-EE suggesting a beneficial effect of EPA and DHA combined as TAG. This finding suggests that EPA and DHA activate the efflux of glycerol from the cells which leads to a decrease in lipid droplets size. Curiously, the gene expression of *AQP7* was kept unchanged in all three adipose tissues (there was no fat depot effect) confirming the conservative role in glycerol release from adipocytes that ensure lipid homeostasis.

ADIPO has emerged as the most abundant adipocyte product, thereby redefining adipose tissue as a key component of the endocrine system (Cao, 2014). In obesity, the circulating levels of *ADIPO* fall while *LEP* levels rise, suggesting that the regulation of these two adipocyte derived hormones may be simultaneously influenced by common obesity related factors (Bastard et al., 2006). The regulation pattern of gene expression of these two adipokines in subcutaneous and visceral WAT were similar, perhaps subjective to common control of energy balance. This interpretation is in line with the positive correlation found between these adipokines in subcutaneous WAT, but not in visceral WAT. However, in BAT both *ADIPO* and *LEP* were down-regulated indicating that ADIPO and LEP have in WAT their major contributor.

Conversely, *PPARα* is highly expressed in BAT which certainly relates to fatty acid β-oxidation (Kersten et al., 2000) occurring in brown adipocytes since BAT is primarily a thermogenic organ. In turn, *PPARγ* stimulates glycerol transport, and glycerol phosphorylation (Guan et al., 2002; Tordjman et al., 2003). *AQP7* is a direct *PPARγ* target gene in adipocytes (Kishida et al., 2001; Guan et al., 2002). This evidence is supported by the moderate positive correlation found between *AQP7* and *PPARγ* mRNA levels in BAT, but not in WAT. Furthermore, we have demonstrated that *PPARα* and *PPARγ* expression correlate significantly in subcutaneous WAT, with the expression of *GLUT4*, an essential gene in the lipogenesis pathway. These findings demonstrate a relationship between *PPARs* expression and the expression of others genes of lipid metabolism, and support the hypothesis that *PPARα* and *PPARγ* activators may regulate fatty acid metabolism in adipose tissue.

Variations of response across fat depots (subcutaneous WAT, visceral WAT and BAT) suggest that the features of adipose tissue responsible for adipocyte cells and differentiation may not be homogenous, both in nature and distribution, as proven by the discriminant

analysis (PCA) herein presented. Visceral fat accumulation, as supported by *PPARγ* upregulation which is the key regulator of adipocytes differentiation, is important in clinical terms because it is more closely linked to the metabolic syndrome due to its inflammatory features. Conversely, brown adipocytes constitute a metabolically active tissue responsible for non-shivering thermogenesis, depletion of excess calories being less inflammatory than WAT. These features are in line with *PPARα*, *ADIPO* and *LEP* genes variations. BAT is associated with metabolic health due to its oxidative capacity and appears to be effective in the protection against metabolic disorders associated with obesity and diabetes.

Moreover, the structure of combined EPA and DHA as EE reduced most of mRNA expression. It is the case of *ADIPO*, *GLUT4* and *PPARγ* in subcutaneous WAT as well as *ADIPO* and *LEP* in BAT. Based on current research, the physiological activity of *n*-3 PUFA and in particular, the potential effect of a specific fatty acid depends both on its structure and administration form. There seems to be a difference in the apparent bioavailability of *n*-3 PUFA, such that the TAG form is more bioavailable than the EE form (Lawson & Hughes, 1988a; Lawson & Hughes, 1988b). This interpretation is in line with our own findings. TAG define three fatty acid esterified (bonded) to a glycerol backbone and is the natural molecular form that make up virtually all fats and oils in both animal and plants species, devoided of toxicological effects and more chemically stable than EE. The EE form lacks the glycerol backbone needed to reassemble the TAG structure. Some experts have postulated that the lack of glycerol backbone is the major obstacle to the efficient absorption of EE form EPA and DHA.

In summary, in this work:

1) we found differential *AQP3*, *AQP5* and *AQP7* gene expression across subcutaneous WAT, visceral WAT and BAT, which might reflect adipose tissue depot's own location and metabolic function;

2) we found different patterns of aquaporins expression between WAT (subcutaneous and visceral) and BAT when hamsters were fed on specific *n*-3 PUFA molecular structures: FO (rich in EPA and DHA in the TAG form) and FO-EE (rich in EPA and DHA in the EE form) *versus* linseed oil (LSO rich in ALA, 18:3*n*-3);

3) we further complemented this study with the transcriptional profile of lipid sensitive mediators, namely *ADIPO*, *LEP*, *GLUT4*, *PPARα* and *PPARγ* across subcutaneous WAT, visceral WAT and BAT and concluded the same outcome as described in point **2**;

4) we established that BAT is more sensitive to *n*-3 PUFA molecular lipid structures than WAT (both subcutaneous or visceral);

5) with minor exceptions, the FO diet, in opposition to LSO and FO-EE, upregulated almost all players (aquaporins and lipid sensitive mediators) of fat balance and energy homeostasis, highlighting the nutritional benefits of EPA and DHA combined as TAG;

6) we concluded, that among the tested aquaporins isoforms, the aquaglyceroporin *AQP7* stands out as the more promising target for developing new anti-obesity drugs due to its conservative role across WAT and BAT.

7. References

Abbott SK, Else PL, Atkins TA, Hulbert AJ (2012) Fatty acid composition of membrane bilayers: importance of diet polyunsaturated fat balance. *Biochim Biophys Acta* **1818:** 1309-1317

Agre P (2004) Aquaporin water channels (Nobel Lecture). *Angew Chem Int Ed Engl* **43:** 4278-4290

AOAC (1995) AOAC official methods of analysis. *AOACS, Washington DC, USA*

Bandarra NM, Batista I, Nunes ML, Empis JM, Christie WW (1997) Seasonal Changes in Lipid Composition of Sardine (Sardina pilchardus). *Journal of Food Science* **62:** 40–42

Bandarra NM, Lopes PA, Martins SV, Ferreira J, Alfaia CM, Rolo EA, Correia JJ, Pinto RM, Ramos-Bueno RP, Batista I, Prates JA, Guil-Guerrero JL (2016) Docosahexaenoic acid at the sn-2 position of structured triacylglycerols improved n-3 polyunsaturated fatty acid assimilation in tissues of hamsters. *Nutrition Research* **36:** 452-463

Bastard JP, Maachi M, Lagathu C, Kim MJ, Caron M, Vidal H, Capeau J, Feve B (2006) Recent advances in the relationship between obesity, inflammation, and insulin resistance. *European cytokine network* **17:** 4-12

Benga G (2012) On the definition, nomenclature and classification of water channel proteins (aquaporins and relatives). *Molecular aspects of medicine* **33:** 514-517

Berry DC, Stenesen D, Zeve D, Graff JM (2013) The developmental origins of adipose tissue. *Development* **140:** 3939-3949

Beynen AC, Hermus RJ, Hautvast JG (1980) A mathematical relationship between the fatty acid composition of the diet and that of the adipose tissue in man. *Am J Clin Nutr* **33:** 81-85

Bezard J, Blond JP, Bernard A, Clouet P (1994) The metabolism and availability of essential fatty acids in animal and human tissues. *Reproduction, nutrition, development* **34:** 539-568

Biong AS, Veierod MB, Ringstad J, Thelle DS, Pedersen JI (2006) Intake of milk fat, reflected in adipose tissue fatty acids and risk of myocardial infarction: a case-control study. *European journal of clinical nutrition* **60:** 236-244

Braga M, Reddy ST, Vergnes L, Pervin S, Grijalva V, Stout D, David J, Li X, Tomasian V, Reid CB, Norris KC, Devaskar SU, Reue K, Singh R (2014) Follistatin promotes adipocyte differentiation, browning, and energy metabolism. *Journal of lipid research* **55:** 375-384

Buckley JD, Howe PR (2010) Long-chain omega-3 polyunsaturated fatty acids may be beneficial for reducing obesity-a review. *Nutrients* **2:** 1212-1230

Calder PC (2012) Mechanisms of action of (n-3) fatty acids. *The Journal of nutrition* **142:** 592-599

Campos-Silva P, Furriel A, Costa WS, Sampaio FJ, Gregorio BM (2015) Metabolic and testicular effects of the long-term administration of different high-fat diets in adult rats. *International Braz J urol: Official Journal of the Brazilian Society of Urology* **41:** 569-575

Cannon B, Nedergaard J (2004) Brown adipose tissue: function and physiological significance. *Physiological reviews* **84:** 277-359

Cao H (2014) Adipocytokines in obesity and metabolic disease. *The Journal of endocrinology* **220:** 47-59

Capurso C, Capurso A (2012) From excess adiposity to insulin resistance: the role of free fatty acids. *Vascular pharmacology* **57:** 91-97

Carbrey JM, Agre P (2009) Discovery of the aquaporins and development of the field. *Handbook of experimental pharmacology* **190:** 3-28

Christie WW (1989) Preparation of methyl ester and other derivatives. *WW Christie (Ed), Gas chromatography and lipids: a practical guide, Pergamon Press, New York, USA***:** 64-84

Cohen P, Yang G, Yu X, Soukas AA, Wolfish CS, Friedman JM, Li C (2005) Induction of leptin receptor expression in the liver by leptin and food deprivation. *The Journal of biological chemistry* **280:** 10034-10039

Corvera S, Gealekman O (2014) Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. *Biochim Biophys Acta* **1842:** 463-472

Covaci A, Voorspoels S, Thomsen C, van Bavel B, Neels H (2006) Evaluation of total lipids using enzymatic methods for the normalization of persistent organic pollutant levels in serum. *Sci Total Environ* **366:** 361-366

Cowherd RM, Lyle RE, McGehee RE, Jr. (1999) Molecular regulation of adipocyte differentiation. *Seminars in cell & developmental biology* **10:** 3-10

Crupi R, Marino A, Cuzzocrea S (2013) n-3 fatty acids: role in neurogenesis and neuroplasticity. *Current medicinal chemistry* **20:** 2953-2963

Cypess AM, Lehman S, Williams G, Tal I, Rodman D, Goldfine AB, Kuo FC, Palmer EL, Tseng YH, Doria A, Kolodny GM, Kahn CR (2009) Identification and importance of brown adipose tissue in adult humans. *The New England journal of medicine* **360:** 1509-1517

da Silva IV, Soveral G (2017) Aquaporins in Obesity. *Advances in experimental medicine and biology* **969:** 227-238

Dalboge LS, Pedersen PJ, Hansen G, Fabricius K, Hansen HB, Jelsing J, Vrang N (2015) A Hamster Model of Diet-Induced Obesity for Preclinical Evaluation of Anti-Obesity, Anti-Diabetic and Lipid Modulating Agents. *PLoS One* **10:** 634-648

Das UN, Fams (2003) Long-chain polyunsaturated fatty acids in the growth and development of the brain and memory. *Nutrition* **19:** 62-65

De Pauw A, Tejerina S, Raes M, Keijer J, Arnould T (2009) Mitochondrial (dys)function in adipocyte (de)differentiation and systemic metabolic alterations. *Am J Pathol* **175:** 927-939

de Souza RJ, Mente A, Maroleanu A, Cozma AI, Ha V, Kishibe T, Uleryk E, Budylowski P, Schunemann H, Beyene J, Anand SS (2015) Intake of saturated and trans unsaturated fatty acids and risk of all cause mortality, cardiovascular disease, and type 2 diabetes: systematic review and meta-analysis of observational studies. *Bmj (clinical research ed)* **351:** 3978-3994

Douris N, Stevanovic DM, Fisher FM, Cisu TI, Chee MJ, Nguyen NL, Zarebidaki E, Adams AC, Kharitonenkov A, Flier JS, Bartness TJ, Maratos-Flier E (2015) Central Fibroblast Growth Factor 21 Browns White Fat via Sympathetic Action in Male Mice. *Endocrinology* **156:** 2470-2481

Dyall SC (2015) Long-chain omega-3 fatty acids and the brain: a review of the independent and shared effects of EPA, DPA and DHA. *Frontiers in aging neuroscience* **7:** 52-67

Estadella D, da Penha Oller do Nascimento CM, Oyama LM, Ribeiro EB, Damaso AR, de Piano A (2013) Lipotoxicity: effects of dietary saturated and transfatty acids. *Mediators of inflammation* **Doi: 10.1155/2013/137579**

Fantuzzi G (2005) Adipose tissue, adipokines, and inflammation. *The Journal of allergy and clinical immunology* **115:** 911-920

Fischer G, Kosinska-Eriksson U, Aponte-Santamaria C, Palmgren M, Geijer C, Hedfalk K, Hohmann S, de Groot BL, Neutze R, Lindkvist-Petersson K (2009) Crystal structure of a yeast aquaporin at 1.15 angstrom reveals a novel gating mechanism. *PLoS biology* **7:** 130-143

Fleige S, Pfaffl MW (2006) RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular aspects of medicine* **27:** 126-139

Fleige S, Walf V, Huch S, Prgomet C, Sehm J, Pfaffl MW (2006) Comparison of relative mRNA quantification models and the impact of RNA integrity in quantitative real-time RT-PCR. *Biotechnol Lett* **28:** 1601-1613

Friedewald WT, Levy RI, Fredrickson DS (1972) Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. *Clin Chem* **18:** 499-502

Fruhbeck G (2005) Obesity: aquaporin enters the picture. *Nature* **438:** 436-438

Fruhbeck G, Catalan V, Gomez-Ambrosi J, Rodriguez A (2006) Aquaporin-7 and glycerol permeability as novel obesity drug-target pathways. *Trends in pharmacological sciences* **27:** 345-347

Fu D, Libson A, Miercke LJ, Weitzman C, Nollert P, Krucinski J, Stroud RM (2000) Structure of a glycerol-conducting channel and the basis for its selectivity. *Science* **290:** 481-486

Fu Y, Luo N, Klein RL, Garvey WT (2005) Adiponectin promotes adipocyte differentiation, insulin sensitivity, and lipid accumulation. *Journal of lipid research* **46:** 1369-1379

Gonen T, Cheng Y, Sliz P, Hiroaki Y, Fujiyoshi Y, Harrison SC, Walz T (2005) Lipid-protein interactions in double-layered two-dimensional AQP0 crystals. *Nature* **438:** 633-638

Griffin BA (2008) How relevant is the ratio of dietary n-6 to n-3 polyunsaturated fatty acids to cardiovascular disease risk? Evidence from the OPTILIP study. *Current opinion in lipidology* **19:** 57-62

Grosso G, Galvano F, Marventano S, Malaguarnera M, Bucolo C, Drago F, Caraci F (2014) Omega-3 fatty acids and depression: scientific evidence and biological mechanisms. *Oxidative medicine and cellular longevity* **2014:** 70-86

Guan HP, Li Y, Jensen MV, Newgard CB, Steppan CM, Lazar MA (2002) A futile metabolic cycle activated in adipocytes by antidiabetic agents. *Nature medicine* **8:** 1122-1128

Hansen IR, Jansson KM, Cannon B, Nedergaard J (2014) Contrasting effects of cold acclimation versus obesogenic diets on chemerin gene expression in brown and brite adipose tissues. *Biochim Biophys Acta* **1841:** 1691-1699

Hardy OT, Czech MP, Corvera S (2012) What causes the insulin resistance underlying obesity? *Current opinion in endocrinology, diabetes, and obesity* **19:** 81-87

Harms M, Seale P (2013) Brown and beige fat: development, function and therapeutic potential. *Nature medicine* **19:** 1252-1263

Haupt A, Thamer C, Heni M, Machicao F, Machann J, Schick F, Stefan N, Fritsche A, Haring HU, Staiger H (2010) Novel obesity risk loci do not determine distribution of body fat depots: a whole-body MRI/MRS study. *Obesity* **18:** 1212-1217

Hinrichsen N (2016) Commercially available alternatives to palm oil. *Lipid technology* **28:** 65-67

Hirsch J, Farquhar JW, Ahrens EH, Jr., Peterson ML, Stoffel W (1960) Studies of adipose tissue in man. A microtechnic for sampling and analysis. *Am J Clin Nutr* **8:** 499-511

Hodson L, Skeaff CM, Fielding BA (2008) Fatty acid composition of adipose tissue and blood in humans and its use as a biomarker of dietary intake. *Prog Lipid Res* **47:** 348-380

Holness MJ, Greenwood GK, Smith ND, Sugden MC (2003) Diabetogenic impact of long-chain omega-3 fatty acids on pancreatic beta-cell function and the regulation of endogenous glucose production. *Endocrinology* **144:** 3958-3968

Horsefield R, Norden K, Fellert M, Backmark A, Tornroth-Horsefield S, Terwisscha van Scheltinga AC, Kvassman J, Kjellbom P, Johanson U, Neutze R (2008) High-resolution x-ray structure of human aquaporin 5. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 13327-13332

Hub JS, de Groot BL (2008) Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proceedings of the National Academy of Sciences of the United States of America* **105:** 1198-1203

Hub JS, Grubmuller H, de Groot BL (2009) Dynamics and energetics of permeation through aquaporins. What do we learn from molecular dynamics simulations? *Handbook of experimental pharmacology***:** 57-76

Hunter JE (2001) Studies on effects of dietary fatty acids as related to their position on triglycerides. *Lipids* **36:** 655-668

Husted KS, Bouzinova EV (2016) The importance of n-6/n-3 fatty acids ratio in the major depressive disorder. *Medicina* **52:** 139-147

Huth PJ, Park KM (2012) Influence of dairy product and milk fat consumption on cardiovascular disease risk: a review of the evidence. *Advances in nutrition* **3:** 266-285

Imran M, Nadeem M (2015) Triacylglycerol composition, physico-chemical characteristics and oxidative stability of interesterified canola oil and fully hydrogenated cottonseed oil blends. *Lipids in health and disease* **14:** 138-149

Ishida T, Ohta M, Nakakuki M, Kami H, Uchiyama R, Kawano H, Notsu T, Imada K, Shimano H (2013) Distinct regulation of plasma LDL cholesterol by eicosapentaenoic acid and docosahexaenoic acid in high fat diet-fed hamsters: participation of cholesterol ester transfer protein and LDL receptor. *Prostaglandins, leukotrienes, and essential fatty acids* **88:** 281-288

Itah R, Gitelman I, Davis C (2004) A replacement for methoxyflurane (Metofane) in open-circuit anaesthesia. *Lab Anim* **38:** 280-285

Joris PJ, Mensink RP (2016) Role of cis-Monounsaturated Fatty Acids in the Prevention of Coronary Heart Disease. *Current atherosclerosis reports* **18:** 38-45

Jung JS, Preston GM, Smith BL, Guggino WB, Agre P (1994) Molecular structure of the water channel through aquaporin CHIP. The hourglass model. *The Journal of biological chemistry* **269:** 14648-14654

Kadegowda AK, Piperova LS, Erdman RA (2008) Principal component and multivariate analysis of milk long-chain fatty acid composition during diet-induced milk fat depression. *Journal of dairy science* **91:** 749-759

Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, Quon MJ (2000) Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. *The Journal of clinical endocrinology and metabolism* **85:** 2402-2410

Kersten S, Desvergne B, Wahli W (2000) Roles of PPARs in health and disease. *Nature* **405:** 421-424

Khandelwal S, Kelly L, Malik R, Prabhakaran D, Reddy S (2013) Impact of omega-6 fatty acids on cardiovascular outcomes: A review. *Journal of preventive cardiology* **2:** 325-336

Kishida K, Kuriyama H, Funahashi T, Shimomura I, Kihara S, Ouchi N, Nishida M, Nishizawa H, Matsuda M, Takahashi M, Hotta K, Nakamura T, Yamashita S, Tochino Y, Matsuzawa Y (2000) Aquaporin adipose, a putative glycerol channel in adipocytes. *The Journal of biological chemistry* **275:** 20896-20902

Kishida K, Shimomura I, Nishizawa H, Maeda N, Kuriyama H, Kondo H, Matsuda M, Nagaretani H, Ouchi N, Hotta K, Kihara S, Kadowaki T, Funahashi T, Matsuzawa Y (2001) Enhancement of the aquaporin adipose gene expression by a peroxisome proliferator-activated receptor gamma. *The Journal of biological chemistry* **276:** 48572-48579

Kondreddy VK, Anikisetty M, Naidu KA (2016) Medium-chain triglycerides and monounsaturated fatty acids potentiate the beneficial effects of fish oil on selected cardiovascular risk factors in rats. *The Journal of nutritional biochemistry* **28:** 91-102

Kremmyda LS, Tvrzicka E, Stankova B, Zak A (2011) Fatty acids as biocompounds: their role in human metabolism, health and disease: a review. part 2: fatty acid physiological roles and applications in human health and disease. *Biomedical papers of the Medical Faculty of the University Palacky, Olomouc, Czechoslovakia* **155:** 195-218

Labbe SM, Caron A, Bakan I, Laplante M, Carpentier AC, Lecomte R, Richard D (2015) In vivo measurement of energy substrate contribution to cold-induced brown adipose tissue thermogenesis. *FASEB J* **29:** 2046-2058

Laforest S, Labrecque J, Michaud A, Cianflone K, Tchernof A (2015) Adipocyte size as a determinant of metabolic disease and adipose tissue dysfunction. *Critical reviews in clinical laboratory sciences* **52:** 301-313

Lawson LD, Hughes BG (1988a) Absorption of eicosapentaenoic acid and docosahexaenoic acid from fish oil triacylglycerols or fish oil ethyl esters co-ingested with a high-fat meal. *Biochemical and biophysical research communications* **156:** 960-963

Lawson LD, Hughes BG (1988b) Human absorption of fish oil fatty acids as triacylglycerols, free acids, or ethyl esters. *Biochemical and biophysical research communications* **152:** 328-335

Lebeck J (2014) Metabolic impact of the glycerol channels AQP7 and AQP9 in adipose tissue and liver. *Journal of molecular endocrinology* **52:** 165-178

Lee J, Chung DS, Kang JH, Yu BY (2012) Comparison of visceral fat and liver fat as risk factors of metabolic syndrome. *Journal of Korean medical science* **27:** 184-189

Lewinska A, Zebrowski J, Duda M, Gorka A, Wnuk M (2015) Fatty Acid Profile and Biological Activities of Linseed and Rapeseed Oils. *Molecules* **20:** 22872-22880

Li A, Li KX, Marui S, Krozowski ZS, Batista MC, Whorwood CB, Arnhold IJ, Shackleton CH, Mendonca BB, Stewart PM (1997) Apparent mineralocorticoid excess in a Brazilian kindred: hypertension in the heterozygote state. *Journal of hypertension* **15:** 1397-1402

Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* **25:** 402-408

Lopes PA, Bandarra NM, Martins SV, Madeira MS, Ferreira J, Guil-Guerrero JL, Prates JAM (2017) Docosahexaenoic acid (DHA) at the sn-2 position of triacylglycerols increases DHA incorporation in brown, but not in white adipose tissue, of hamsters. *International journal of food sciences and nutrition* **DOI: 10.1080/09637486.2017.1372390.**

Lowell BB, Flier JS (1997) Brown adipose tissue, beta 3-adrenergic receptors, and obesity. *Annual review of medicine* **48:** 307-316

Ludescher B, Najib A, Baar S, Machann J, Thamer C, Schick F, Buchkremer G, Claussen CD, Eschweiler GW (2007) Gender specific correlations of adrenal gland size and body fat distribution: a whole body MRI study. *Hormone and metabolic research* **39:** 515-518

Madeira A, Fernandez-Veledo S, Camps M, Zorzano A, Moura TF, Ceperuelo-Mallafre V, Vendrell J, Soveral G (2014) Human aquaporin-11 is a water and glycerol channel and localizes in the vicinity of lipid droplets in human adipocytes. *Obesity* **22:** 2010-2017

Madeira A, Mosca AF, Moura TF, Soveral G (2015a) Aquaporin-5 is expressed in adipocytes with implications in adipose differentiation. *IUBMB Life* **67:** 54-60

Madeira A, Moura TF, Soveral G (2015b) Aquaglyceroporins: implications in adipose biology and obesity. *Cellular and molecular life sciences* **72:** 759-771

Maeda N, Funahashi T, Shimomura I (2008) Metabolic impact of adipose and hepatic glycerol channels aquaporin 7 and aquaporin 9. *Nature clinical practice Endocrinology & metabolism* **4:** 627-634

Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC (1985) Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* **28:** 412-419

Mensink RP, Zock PL, Kester AD, Katan MB (2003) Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am J Clin Nutr* **77:** 1146-1155

Mercer SW, Trayhurn P (1987) Effect of high fat diets on energy balance and thermogenesis in brown adipose tissue of lean and genetically obese ob/ob mice. *The Journal of nutrition* **117:** 2147-2153

Micha R, Mozaffarian D (2010) Saturated fat and cardiometabolic risk factors, coronary heart disease, stroke, and diabetes: a fresh look at the evidence. *Lipids* **45:** 893-905

Miranda M, Escote X, Ceperuelo-Mallafre V, Alcaide MJ, Simon I, Vilarrasa N, Wabitsch M, Vendrell J (2010) Paired subcutaneous and visceral adipose tissue aquaporin-7 expression in human obesity and type 2 diabetes: differences and similarities between depots. *The Journal of Clinical Endocrinology and Metabolism* **95:** 3470-3479

Molfino A, Gioia G, Rossi Fanelli F, Muscaritoli M (2014) The role for dietary omega-3 fatty acids supplementation in older adults. *Nutrients* **6:** 4058-4073

Mori TA, Watts GF, Burke V, Hilme E, Puddey IB, Beilin LJ (2000) Differential effects of eicosapentaenoic acid and docosahexaenoic acid on vascular reactivity of the forearm microcirculation in hyperlipidemic, overweight men. *Circulation* **102:** 1264-1269

Murata K, Mitsuoka K, Hirai T, Walz T, Agre P, Heymann JB, Engel A, Fujiyoshi Y (2000) Structural determinants of water permeation through aquaporin-1. *Nature* **407:** 599-605

Murphy KT, Schwartz GJ, Nguyen NL, Mendez JM, Ryu V, Bartness TJ (2013) Leptin-sensitive sensory nerves innervate white fat. *American journal of physiology Endocrinology and metabolism* **304:** 1338-1347

Nedergaard J, Becker W, Cannon B (1983) Effects of dietary essential fatty acids on active thermogenin content in rat brown adipose tissue. *The Journal of nutrition* **113:** 1717-1724

Nedergaard J, Bengtsson T, Cannon B (2007) Unexpected evidence for active brown adipose tissue in adult humans. *American journal of physiology Endocrinology and metabolism* **293:** 444-452

Nedergaard J, Cannon B (2010) The changed metabolic world with human brown adipose tissue: therapeutic visions. *Cell metabolism* **11:** 268-272

O'Sullivan TA, Hafekost K, Mitrou F, Lawrence D (2013) Food sources of saturated fat and the association with mortality: a meta-analysis. *American journal of public health* **103:** 31-42

Orsavova J, Misurcova L, Ambrozova JV, Vicha R, Mlcek J (2015) Fatty Acids Composition of Vegetable Oils and Its Contribution to Dietary Energy Intake and Dependence of Cardiovascular Mortality on Dietary Intake of Fatty Acids. *International journal of molecular sciences* **16:** 12871-12890

Park A, Kim WK, Bae KH (2014) Distinction of white, beige and brown adipocytes derived from mesenchymal stem cells. *World journal of stem cells* **6:** 33-42

Park HT, Lee ES, Cheon YP, Lee DR, Yang KS, Kim YT, Hur JY, Kim SH, Lee KW, Kim T (2012) The relationship between fat depot-specific preadipocyte differentiation and metabolic syndrome in obese women. *Clinical endocrinology* **76:** 59-66

Peschechera A, Eckel J (2013) "Browning" of adipose tissue--regulation and therapeutic perspectives. *Arch Physiol Biochem* **119:** 151-160

Rao RR, Long JZ, White JP, Svensson KJ, Lou J, Lokurkar I, Jedrychowski MP, Ruas JL, Wrann CD, Lo JC, Camera DM, Lachey J, Gygi S, Seehra J, Hawley JA, Spiegelman BM (2014) Meteorin-like is a hormone that regulates immune-adipose interactions to increase beige fat thermogenesis. *Cell* **157:** 1279-1291

Rosen ED, MacDougald OA (2006) Adipocyte differentiation from the inside out. *Nature reviews Molecular cell biology* **7:** 885-896

Rosen ED, Sarraf P, Troy AE, Bradwin G, Moore K, Milstone DS, Spiegelman BM, Mortensen RM (1999) PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Molecular cell* **4:** 611-617

Ross BM, Babay S, Malik I (2015) Brain and Liver Headspace Aldehyde Concentration Following Dietary Supplementation with n-3 Polyunsaturated Fatty Acids. *Lipids* **50:** 1123-1131

Rothwell NJ, Stock MJ (1979) A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281:** 31-35

Saely CH, Geiger K, Drexel H (2012) Brown versus white adipose tissue: a mini-review. *Gerontology* **58:** 15-23

Saito M, Okamatsu-Ogura Y, Matsushita M, Watanabe K, Yoneshiro T, Nio-Kobayashi J, Iwanaga T, Miyagawa M, Kameya T, Nakada K, Kawai Y, Tsujisaki M (2009) High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* **58:** 1526-1531

Savage DF, Egea PF, Robles-Colmenares Y, O'Connell JD, 3rd, Stroud RM (2003) Architecture and selectivity in aquaporins: 2.5 a X-ray structure of aquaporin Z. *PLoS biology* **1:** 334-340

Seidell JC, Oosterlee A, Deurenberg P, Hautvast JG, Ruijs JH (1988) Abdominal fat depots measured with computed tomography: effects of degree of obesity, sex, and age. *European journal of clinical nutrition* **42:** 805-815

Simopoulos AP (2006) Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: nutritional implications for chronic diseases. *Biomedicine & pharmacotherapy* **60:** 502-507

Simopoulos AP (2008) The omega-6/omega-3 fatty acid ratio, genetic variation, and cardiovascular disease. *Asia Pacific journal of clinical nutrition* **17** 131-134

Siri-Tarino PW, Chiu S, Bergeron N, Krauss RM (2015) Saturated Fats Versus Polyunsaturated Fats Versus Carbohydrates for Cardiovascular Disease Prevention and Treatment. *Annual review of nutrition* **35:** 517-543

Spalding KL, Arner E, Westermark PO, Bernard S, Buchholz BA, Bergmann O, Blomqvist L, Hoffstedt J, Naslund E, Britton T, Concha H, Hassan M, Ryden M, Frisen J, Arner P (2008) Dynamics of fat cell turnover in humans. *Nature* **453:** 783-787

Stern JH, Rutkowski JM, Scherer PE (2016) Adiponectin, Leptin, and Fatty Acids in the Maintenance of Metabolic Homeostasis through Adipose Tissue Crosstalk. *Cell metabolism* **23:** 770-784

Sui H, Han BG, Lee JK, Walian P, Jap BK (2001) Structural basis of water-specific transport through the AQP1 water channel. *Nature* **414:** 872-878

Swinburn BA, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL (2011) The global obesity pandemic: shaped by global drivers and local environments. *Lancet* **378:** 804-814

Taha AY, Cheon Y, Faurot KF, Macintosh B, Majchrzak-Hong SF, Mann JD, Hibbeln JR, Ringel A, Ramsden CE (2014) Dietary omega-6 fatty acid lowering increases bioavailability of omega-3 polyunsaturated fatty acids in human plasma lipid pools. *Prostaglandins, leukotrienes, and essential fatty acids* **90:** 151-157

Thomas EL, Saeed N, Hajnal JV, Brynes A, Goldstone AP, Frost G, Bell JD (1998) Magnetic resonance imaging of total body fat. *Journal of applied physiology* **85:** 1778-1785

Todorcevic M, Hodson L (2015) The Effect of Marine Derived n-3 Fatty Acids on Adipose Tissue Metabolism and Function. *Journal of clinical medicine* **5:** 3-27

Tordjman J, Khazen W, Antoine B, Chauvet G, Quette J, Fouque F, Beale EG, Benelli C, Forest C (2003) Regulation of glyceroneogenesis and phosphoenolpyruvate carboxykinase by fatty acids, retinoic acids and thiazolidinediones: potential relevance to type 2 diabetes. *Biochimie* **85:** 1213-1218

Tornroth-Horsefield S, Wang Y, Hedfalk K, Johanson U, Karlsson M, Tajkhorshid E, Neutze R, Kjellbom P (2006) Structural mechanism of plant aquaporin gating. *Nature* **439:** 688-694

Trayhurn P, Beattie JH (2001) Physiological role of adipose tissue: white adipose tissue as an endocrine and secretory organ. *The Proceedings of the Nutrition Society* **60:** 329-339

Van Gaal LF, Mertens IL, De Block CE (2006) Mechanisms linking obesity with cardiovascular disease. *Nature* **444:** 875-880

van Marken Lichtenbelt WD, Schrauwen P (2011) Implications of nonshivering thermogenesis for energy balance regulation in humans. *Am J Physiol Regul Integr Comp Physiol* **301:** R285-296

Villarroya J, Cereijo R, Villarroya F (2013) An endocrine role for brown adipose tissue? *Am J Physiol Endocrinol Metab* **305:** E567-572

Virtanen KA, Lidell ME, Orava J, Heglind M, Westergren R, Niemi T, Taittonen M, Laine J, Savisto NJ, Enerback S, Nuutila P (2009) Functional brown adipose tissue in healthy adults. *N Engl J Med* **360:** 1518-1525

Wang W, Seale P (2016) Control of brown and beige fat development. *Nature reviews Molecular cell biology* **17:** 691-702

Wang Y, Cohen J, Boron WF, Schulten K, Tajkhorshid E (2007) Exploring gas permeability of cellular membranes and membrane channels with molecular dynamics. *Journal of structural biology* **157:** 534-544

Wang Y, Tajkhorshid E (2010) Nitric Oxide Conduction by the Brain Aquaporin AQP4. *Proteins* **78:** 661-670

Wibrand K, Berge K, Messaoudi M, Duffaud A, Panja D, Bramham CR, Burri L (2013) Enhanced cognitive function and antidepressant-like effects after krill oil supplementation in rats. *Lipids in health and disease* **12:** 6-19

Wilcox G (2005) Insulin and insulin resistance. *The Clinical biochemist Reviews* **26:** 19-39

Williamson JR (1970) Control of energy metabolism in hamster brown adipose tissue. *The Journal of biological chemistry* **245:** 2043-2050

Wu J, Bostrom P, Sparks LM, Ye L, Choi JH, Giang AH, Khandekar M, Virtanen KA, Nuutila P, Schaart G, Huang K, Tu H, van Marken Lichtenbelt WD, Hoeks J, Enerback S, Schrauwen P, Spiegelman BM (2012) Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150:** 366-376

Yang LG, Song ZX, Yin H, Wang YY, Shu GF, Lu HX, Wang SK, Sun GJ (2016) Low n-6/n-3 PUFA Ratio Improves Lipid Metabolism, Inflammation, Oxidative Stress and Endothelial Function in Rats Using Plant Oils as n-3 Fatty Acid Source. *Lipids* **51:** 49-59

Ye L, Wu J, Cohen P, Kazak L, Khandekar MJ, Jedrychowski MP, Zeng X, Gygi SP, Spiegelman BM (2013) Fat cells directly sense temperature to activate thermogenesis. *Proceedings of the National Academy of Sciences of the United States of America* **110:** 12480-12485

Yin W, Carballo-Jane E, McLaren DG, Mendoza VH, Gagen K, Geoghagen NS, McNamara LA, Gorski JN, Eiermann GJ, Petrov A, Wolff M, Tong X, Wilsie LC, Akiyama TE, Chen J, Thankappan A, Xue J, Ping X, Andrews G, Wickham LA, Gai CL, Trinh T, Kulick AA, Donnelly MJ, Voronin GO, Rosa R, Cumiskey AM, Bekkari K, Mitnaul LJ, Puig O, Chen F, Raubertas R, Wong PH, Hansen BC, Koblan KS, Roddy TP, Hubbard BK, Strack AM (2012) Plasma lipid profiling across species for the identification of optimal animal models of human dyslipidemia. *Journal of lipid research* **53:** 51-65

Yin X, Lanza IR, Swain JM, Sarr MG, Nair KS, Jensen MD (2014) Adipocyte mitochondrial function is reduced in human obesity independent of fat cell size. *J Clin Endocrinol Metab* **99:** E209-216

Zhang Y, Li R, Meng Y, Li S, Donelan W, Zhao Y, Qi L, Zhang M, Wang X, Cui T, Yang LJ, Tang D (2014) Irisin stimulates browning of white adipocytes through mitogen-activated protein kinase p38 MAP kinase and ERK MAP kinase signaling. *Diabetes* **63:** 514-525

Zingaretti MC, Crosta F, Vitali A, Guerrieri M, Frontini A, Cannon B, Nedergaard J, Cinti S (2009) The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J* **23:** 3113-3120