ESCOLA DE MEDICINA
PROGRAMA DE PÓS-GRADUAÇÃO EM MEDICINA E CIÊNCIAS DA SAÚDE
ÁREA DE CONCENTRAÇÃO FARMACOLOGIA E BIOQUÍMICA MOLECULAR
DOUTORADO

RAQUEL DAL SASSO FREITAS

AVALIAÇÃO DOS MECANISMOS ENVOLVIDOS NOS EFEITOS DOS ÁCIDOS GRAXOS ÔMEGA-3 SOBRE AS COMPLICAÇÕES ASSOCIADAS AO CÂNCER: UMA INVESTIGAÇÃO FARMACOLÓGICA E BIOQUÍMICA

Porto Alegre
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Orientadora: Profa. Dra. Maria Martha Campos

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À minha família.

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Pesquisa, na sua forma bruta, sempre foi meu dia-a-dia, sem eu me dar conta. Meus pais nos criaram (digo “nos” pois incluo minha irmã) em um ambiente de ciência, leitura e muita curiosidade. Uma das minhas primeiras lembranças é a defesa de doutorado do meu pai, onde eu estava vestida de borboleta e lembro que as bancadas do laboratório (acho que eram as bancadas) pareciam imensas. Outra lembrança que tenho é ir, aos sábados, ler e-mails com a minha mãe na universidade – pois ela sempre trabalhou muito em computadores e nunca soube muito bem explicar o quê. De uma maneira ou de outra, a pesquisa sempre esteve ao meu redor. Desta maneira meio estranha, começo essa página agradecendo a eles, meus pais. Eles me apoiaram muito, de maneira incondicional, nesses últimos quatro anos. Então, meu muito obrigada! Claro que, além dos meus pais, dentro desse universo, existe minha irmã. Uma irmã tão diferente e tão igual ao mesmo tempo. Também, preciso te agradecer por sempre me apoiar, de forma incondicional. E ao Chopp (esclarecendo que é o cachorro), por existir.

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"Down to their innate molecular core, cancer cells are hyperactive, survival-endowed, scrappy, fecund, inventive copies of ourselves."

— Siddhartha Mukherjee (*The Emperor of All Maladies: A Biography of Cancer*)

RESUMO

Os ácidos graxos poli-insaturados ômega-3 possuem propriedades anti-inflamatórias e pró-resolutivas, sendo extensivamente empregados no manejo de doenças crônicas, como o câncer. Nesse caso, podem apresentar efeitos benéficos sobre a progressão das neoplasias, além de prevenirem os efeitos adversos associados ao tratamento quimioterápico. A primeira parte da Tese apresenta uma revisão crítica da literatura sobre os efeitos protetores dos ácidos graxos ômega-3 nas complicações associadas ao câncer, incluindo dor, depressão, síndrome da anorexia-caquexia e síndromes paraneoplásicas. De uma maneira geral, o uso desses ácidos graxos na suplementação de pacientes oncológicos parece muito promissor; todavia, ainda há poucos estudos que investigaram os efeitos clínicos desses nutrientes. Na segunda parte da Tese, foi realizado um trabalho experimental, que investigou a participação dos receptores de ácidos graxos livres, FFA1 e FFA4, em um modelo experimental de caquexia associada ao câncer de pulmão, em camundongos. Como estratégias farmacológicas, foram testados os efeitos do tratamento parenteral com os ácidos graxos de origem natural, ácido α-linolênico (ALA) e ácido docosaexaenoico (DHA). Além disso, foram avaliados os efeitos da administração repetida dos agonistas sintéticos duais dos receptores FFA1/FFA4, GW9508 e TUG891, bem como, dos antagonistas seletivos FFA1 e FFA4, GW1100 e AH7614, respectivamente. A indução de caquexia foi associada com um aumento da expressão dos receptores FFA1 no tecido adiposo e muscular. Foi possível observar que a modulação de ambos os receptores pode representar uma estratégia farmacológica promissora no tratamento da caquexia associada ao câncer, uma vez que o agonista dual FFA1/FFA4, o GW9508, promoveu efeitos benéficos em relação à perda de tecido adiposo, à inflamação, aos déficits comportamentais e às

alterações centrais desses animais, além de reduzir o tamanho tumoral. Cabe ressaltar que o GW9508 apresenta maior afinidade pelos receptores FFA1, em relação ao FFA4, explicando as diferenças em comparação ao TUG891. Ademais, parte dos efeitos favoráveis observados para o ALA e o DHA, sobre diferentes parâmetros relacionados com a caquexia, parecem depender da ativação dos receptores FFA1 e FFA4. Os receptores de membrana FFA1 e FFA4 surgem como alvos farmacológicos interessantes para o manejo da caquexia associada ao câncer, explicando parte dos efeitos benéficos da suplementação com ácidos graxos poli-insaturados ômega-3 em pacientes oncológicos.

Palavras-chave: câncer, caquexia, ômega-3, FFA1, FFA4, GPR40, GPR120

ABSTRACT

Omega-3 fatty acids display anti-inflammatory and pro-resolution properties, being important allies to the treatment of chronic diseases, such as cancer and its complications. Omega-3 can be employed either for preventing the tumour progression or to manage the chemotherapy side effects. The first part brings a critical review about the protective effects of omega-3 fatty acids in cancer-associated complications, such as pain, depression, anorexia-cachexia syndrome, and paraneoplastic syndrome. Overall, the use of these fatty acids in cancer patients that develop complications seems a very interesting approach; however, there is a lack of clinical evidence regarding the use of omega-3 in these patients. The second part presents data from an *in vivo* study that investigated the role of free fatty acid receptors, FFA1 and FFA4, in a mouse model of lung cancer cachexia. As pharmacological strategies, the naturally occurring ligands α-linolenic acid (ALA) and docosahexaenoic acid (DHA), the synthetic dual FFA1/FFA4 agonists GW9508 and TUG891, or the selective FFA1 GW1100 or FFA4 AH7614 antagonists, were administered parenterally to LLC-bearing mice, from seven to 21 days after tumour implantation. The induction of cachexia led to an upregulation of FFA1 receptors in skeletal muscle and adipose tissue. The dual FFA1/FFA4 agonist GW9508 promoted beneficial effects associated to adiposity, inflammation, behavioural deficits, and central alterations in these animals, besides reducing the tumour growth. Remarkably, GW9508 has a 100-fold higher affinity for FFA1 receptors, partly explaining the superior effects in comparison with TUG891. Moreover, part of ALA and DHA effects are mediated by the activation of FFA1 and FFA4 receptors, although additional mechanisms cannot be overlooked. It is possible to conclude that the use of omega-3 fatty acids

remains an important tool in cancer treatment and that FFA1/FFA4 receptors emerge as attractive pharmacological targets for cancer cachexia management.

Keywords: cancer, cachexia, omega-3, FFA1, FFA4, GPR40, GPR120

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1 Fundamentação Teórica

1.1 Ácidos graxos poli-insaturados ômega-3

Os ácidos graxos ômega-3 despertaram interesse científico em decorrência de estudos conduzidos na década de 70, por dois pesquisadores Dinamarqueses, Bang e Dyerberg. Ambos investigaram tanto a dieta, rica em gordura animal proveniente de peixes e baleias, como o perfil lipídico de esquimós da Groenlândia, em virtude da baixa incidência de doenças inflamatórias nessa população (1,2).

Os ácidos graxos ômega-3 são classificados como ácidos graxos de cadeia longa, possuindo de 18 a 22 carbonos e contendo a primeira liga dupla no 3º carbono, a partir do grupamento metil. Dentre os ácidos graxos ômega-3, existem três que se destacam: ácido α-linolênico (ALA), ácido eicosapentaenoico (EPA) e ácido docosaexaenoico (DHA). O ALA, de origem vegetal, pode ser encontrado em sementes e em óleos vegetais, como a linhaça; todavia, no organismo, é convertido em EPA e, posteriormente, em DHA, por reações de elongação e dessaturação (Figura 1 e 2). Já, estes dois últimos, são encontrados em peixes de água fria, como salmão ou sardinha (3).

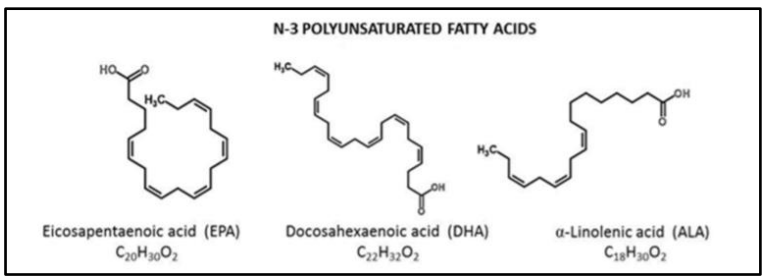

Figura 1 Estrutura química dos ácidos graxos ômega-3. Adaptado de Sokola-Wysoczanska et al (4)

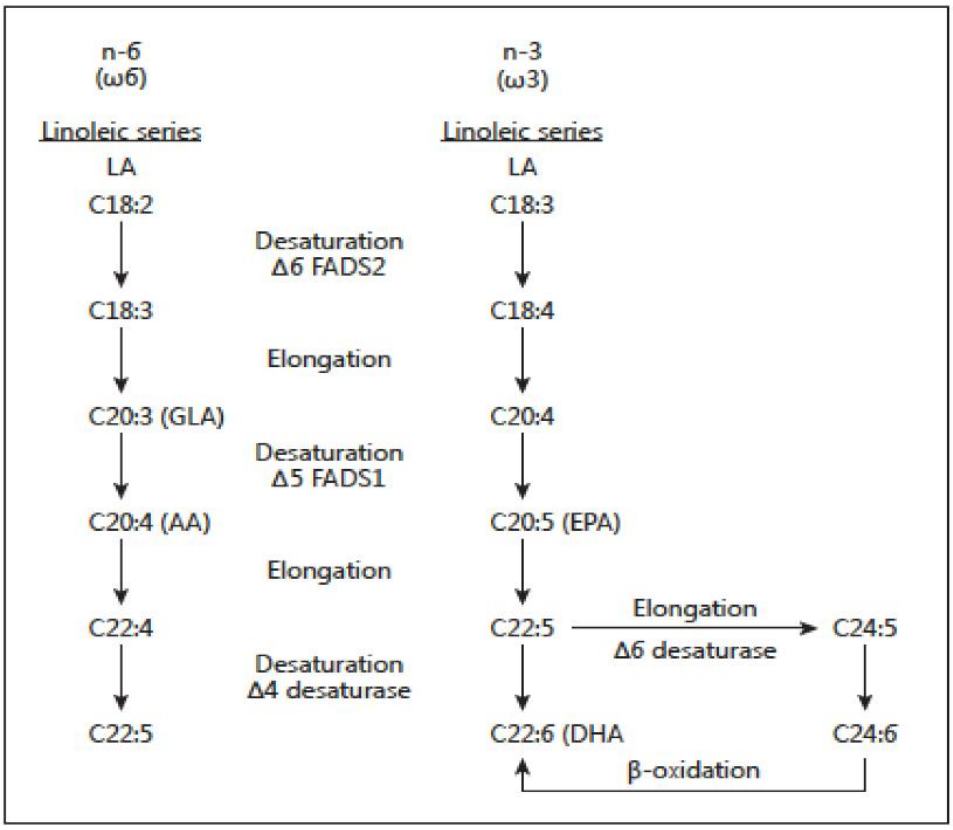

Figura 2 Biossíntese do ácidos graxos poli-insaturados. Retirado de Simopoulos & DiNicolantonio (5)

O alto consumo de óleos ricos em ômega-3 está relacionado à um perfil anti-inflamatório e pró-resolutivo (6). Em contrapartida, os ácidos graxos poli-insaturados ômega-6, principalmente o ácido linoleico (LA), são caracterizados por serem pró-inflamatórios, principalmente por serem precursores do ácido araquidônico (AA), importante componente da membrana plasmática e precursor de mediadores pró-inflamatórios como prostaglandinas e leucotrienos. É importante salientar que o LA é um ácido graxo poli-insaturado essencial que é comumente encontrado em cereais, importante componente da dieta ocidental (5) .

De acordo com Simopoulos (7), um fator importante para altos índices de doenças de origem inflamatória foi o aumento do consumo de alimentos ricos em ômega-6, associado ao desenvolvimento econômico humano, levando à um alto consumo da dieta

ocidental, rica em cereais e óleos vegetais. Após a revolução industrial, ocorreu um decréscimo do consumo de ácidos graxos ômega-3, bem como de vitamina C e E. Por outro lado, o consumo de gordura, principalmente saturada e *trans*, aumentou de forma notável (Figura 3). Adicionalmente, o consumo de ácidos graxos ômega-6 também sofreu este aumento, gerando um aumento na razão ômega-6/ômega-3 de 20:1(5,7).

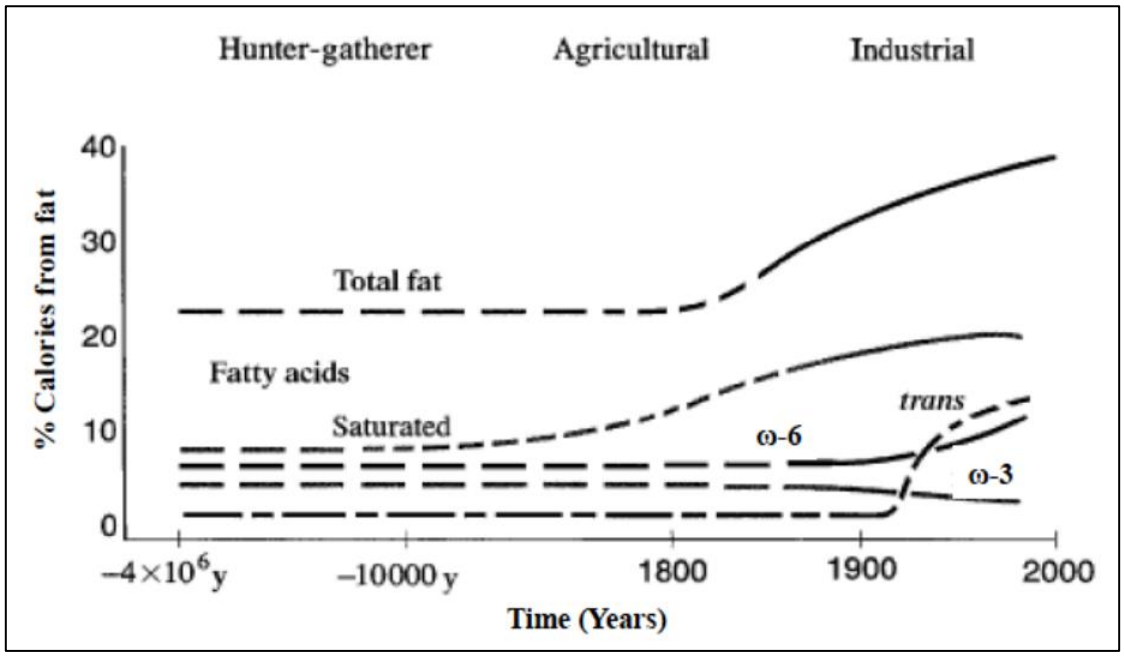

Figura 3 Esquema hipotético do consumo de gordura total e ácidos graxos em percentual de calorias advindas da gordura. Retirado de Simopoulos (7)

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A membrana plasmática contém ALA em pequena quantidade, porém EPA e DHA em quantidades moderadas. De maneira interessante, as membranas celulares cerebrais e oculares apresentam uma alta quantidade de DHA (8). Os ácidos graxos ômega-3 possuem papel importante nas respostas intracelulares por modularem a composição lipídica da membrana plasmática, principalmente, por competir pelas mesmas enzimas que clivam o AA e o LA no processo inflamatório (9). Neste caso, inicialmente, os fosfolipídios de membrana sofrem clivagem pela fosfolipase A2 a fim de

liberar o AA para o citoplasma da célula e, assim, sofrer a ação de cicloxigenases e lipoxigenases, produzindo mediadores inflamatórios, como os eicosanoides. O aumento de moléculas de ômega-3 na composição da membrana plasmática leva à geração de mediadores fracos, como o tromboxano A2 e a prostaciclina I3, a partir da ação das mesmas enzimas (10). Além disso, a quantidade de ômega-3 na membrana celular é de extrema importância para a geração de mediadores pró-resolução da inflamação (11). Corroborando com esse mecanismo, observou-se uma modulação da membrana plasmática e da produção de mediadores pró-inflamatórios em idosos após 1 ano de dieta mediterrânea, cuja principal característica é ser rica em ácidos graxos ômega-3 (12).

Em relação ao processo de resolução da inflamação, os ácidos graxos ômega-3 possuem um papel crucial neste processo. O processo inflamatório se dá, inicialmente, pelo recrutamento de neutrófilos e, posteriormente, das demais células do sistema imune como macrófagos e linfócitos. No processo de inflamação crônica, o excesso de neutrófilos recrutados para o sítio inflamatório podem danificar o local, ocasionalmente entrando em morte celular e liberando ao sítio inflamatório o que havia, anteriormente, sido fagocitado. Em situações em que o processo inflamatório se resolve, que seria na inflação aguda, a resolução da inflamação se dá pela produção de mediadores pró-resolução, como as resolvinas, protectinas/neuroprotectinas e maresinas, que são produzidas a partir dos ácidos graxos ômega-3 (Figura 4).

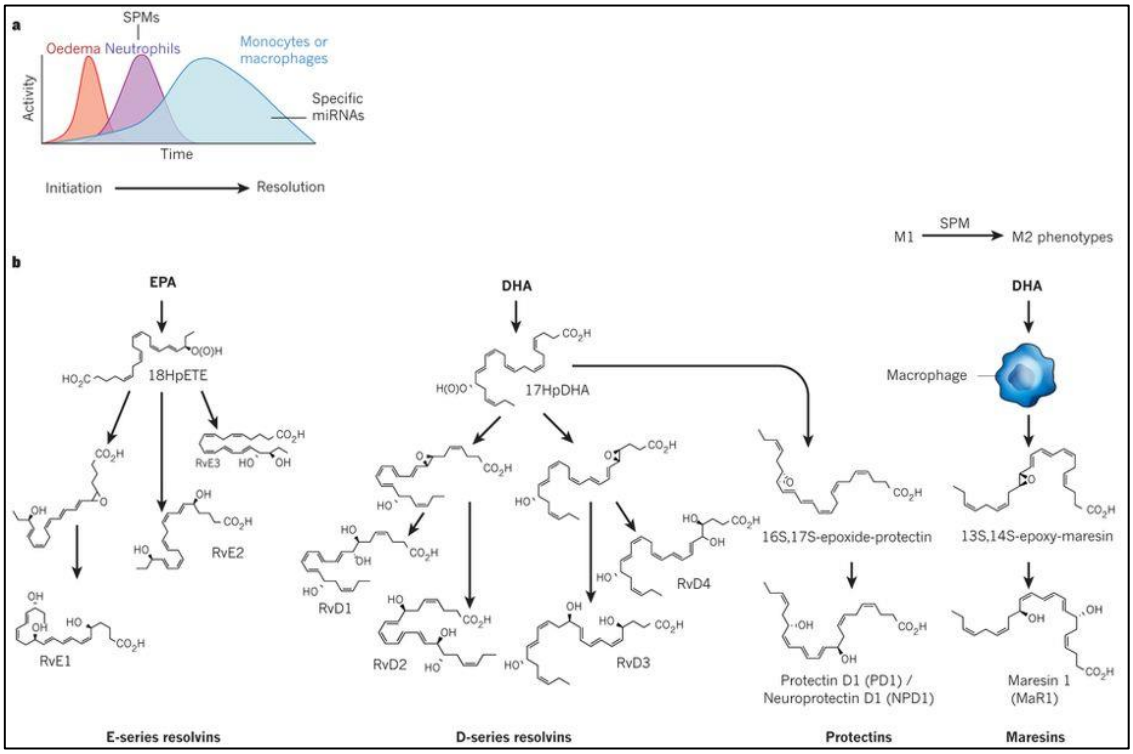

Figura 4 Resposta inflamatória auto limitante e mediadores pró-resolução derivados dos ácidos graxos ômega-3. Retirado de Serhan (13). Licença para uso: 4610990053960

As resolvinas da série E são produzidas a partir do EPA, e as protectinas/neuroprotectinas, resolvinas da série D e maresinas são produzidas a partir do DHA (13,14) Estes mediadores têm sido amplamente investigados como manejo de diferentes patologias. Por exemplo, um estudo do nosso laboratório demonstrou que a resolvina D1 exerce efeitos benéfico sobre a fibromialgia (15). Além disso, já foi previamente demonstrado que tanto a resolvina D1, como a D2 e a E1, podem auxiliar nas terapias antitumorais por estimularem a fagocitose de células tumorais mortas (16).

Tanto os ácidos graxos ômega-3, como seus derivados, apresentam benefícios em diferentes patologias. Atualmente, a suplementação de ômega-3 tem sido amplamente investigada para o manejo de pacientes oncológicos, portadores de doenças cardiovasculares, doenças psiquiátricas, doenças neurológicas e do esporte (17–19).

De maneira interessante, no início do novo milênio, dois receptores acoplados à proteína G foram identificados como alvos farmacológicos dos ácidos graxos livres ômega-3, demonstrando um novo mecanismo de ação para essas moléculas.

1.2 Receptores de ácidos graxos livres

Os receptores de ácidos graxos livres pertencem à família de receptores acoplados à proteína G, com sete domínios transmembrana, sendo ativados de maneira endógena por cadeias de ácidos graxos livres. Sua especificidade depende da quantidade de carbonos do ácido graxo como, por exemplo, o *free fatty acid receptor 1* (FFA1) e o *free fatty acid receptor 4* (FFA4) apresentam maior afinidade por ácidos graxos de cadeia longa (>12 carbonos) (20,21). Já o *free fatty acid receptor 2* (FFA2) e o *free fatty acid receptor 3* (FFA3) demonstram preferência pelos ácidos graxos de cadeia curta (<5 carbonos), sendo esses obtidos através da fermentação das bactérias da microbiota intestinal. Tais receptores representam possíveis alvos farmacológicos para o tratamento de doenças crônicas como diabetes, obesidade, doenças cardiovasculares e doenças inflamatórias intestinais (22,23). É importante salientar que conforme *The Concise Guide to Pharmacology 2017/2018*, a família de receptores de receptores de ácidos graxos ainda é denominada apenas como FFA; porém, a nomenclatura é recente e se pode encontrar variações na literatura com as denominações: GPR40, FFAR1 ou GPR40/FFAR1 (22).

Na Tabela 1, encontram-se os ácidos graxos livres e seus respectivos receptores.

Tabela 1 Afinidade dos ácidos graxos livres pelos receptores FFA1, FFA2, FFA3 e FFA4. Extraída de Miyamoto et al (24).

Ligand	EC_{50} of Ligand Affinity (μM)			
	GPR41/FFAR3	GPR43/FFAR2	GPR40/FFAR1	GPR120/FFAR4
Saturated fatty acids				
acetic acid (C2:0)	>1000 [b–d]	35–431 [b–d]		
propionic acid (C3:0)	6–127 [b–d]	14–290 [b–d]		
butyric acid (C4:0)	42–158 [b–d]	28–371 [b–d]		
valeric acid (C5:0)	42–142 [b–d]	>1000 [b–d]		
caproic acid (C6:0)	102–134 [a,c,d]		46 [a,c,d]	
caprylate (C8:0)			38 [a]	
capric acid (C10:0)			14–43 [a,d]	
lauric acid (C12:0)			6–12 [a,d]	
myristic acid (C14:0)			8–14 [a,d]	30 [a,d]
palmitic acid (C16:0)			5–7 [a,d]	52 [a,d]
stearic acid (C18:0)			17 [a]	18 [a]
Monounsaturated fatty acids				
palmitoleic acid (C16:1, n-7)			14 [a]	0.7–3 [a]
oleic acid (C18:1, n-9)			2–40 [a,d]	31 [a,d]
ω-3 fatty acids				
α-linolenic acid (C18:3, n-3)			2–13 [a,d]	0.5 [a,d]
cis-11,14,17-eicosatrienoic acid (C20:3, n-3)			11 [a]	1 [a]
cis-5,8,11,14,17-eicosapentaenoic acid (C20:5, n-3)			2–7 [a,d]	2–3 [a,d]
docosahexaenoic acid (22:6, n-3)			1–4 [a,d]	4 [a,d]
ω -6 fatty acids				
linoleic acid (C18:2, n-6)			2–10 [a,d]	1 [a,d]
γ-linolenic acid (C18:3, n-6)			5–9 [a,d]	1 [a,d]
dihomo-γ-linolenic acid (C20:4, n-6)			7 [a]	14 [a]
arachidonic acid (C20:4, n-6)			2–12 [a,d]	
docosatetraenoic acid (C22:4, n-6)			13 [a]	16 [a]

Medida pela indução de [Ca2+]i (a) and GTPγs (b) em células HEK293 transfectadas com receptores para FFAs ou cAMP (c) e [Ca2+]i (d) em cada CHO células transfectadas com receptores para FFAs.

1.2.1 Receptor de ácidos graxos livres 1 (FFA1)

O FFA1, anteriormente conhecido como GPR40, foi o primeiro receptor dessa família a ser deorfanizado, após a identificação de agonistas (20). Este receptor é uma

proteína de membrana de 31.45 kDa e possui 300 aminoácidos na sua composição (Figura 5) (20).

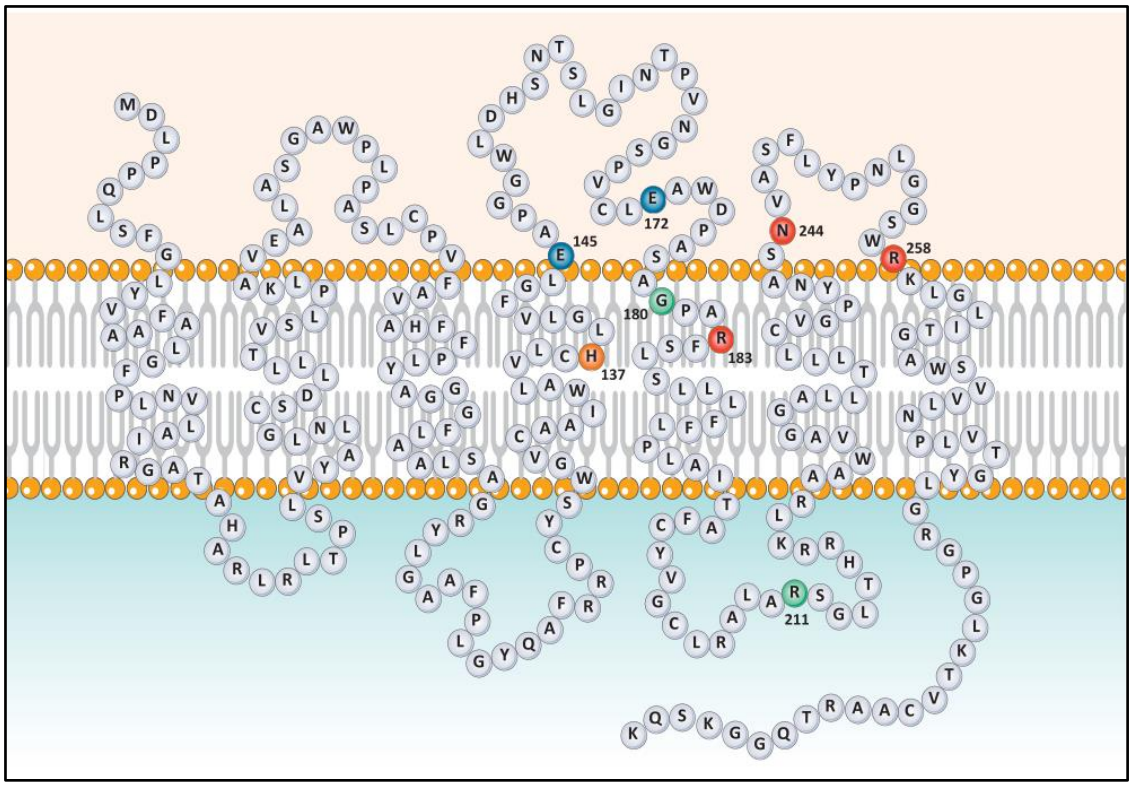

Figura 5 Estrutura do FFA1. Retirada de Mancini & Poitout (25). Licença para uso: 4610981316462

Como descrito na Tabela 1, este receptor pode ser ativado pelos diferentes ácidos graxos, como DHA, ALA, ácido oleico e ácido mirístico (20,22,24). Sua expressão varia de acordo com o tecido, porém sabe-se que os locais de maior expressão do FFA1 são as células β-pancreáticas e o sistema nervoso central (SNC) (25,26). De acordo com Yamashima (2015), esse padrão de expressão do receptor não ocorre ao acaso: neurônios positivos para FFA1 no hipotálamo podem responder diretamente a nutrientes e hormônios como ácidos graxos, glicose e leptina e, podem regular a produção de glicose hepática, a secreção de insulina/glucagon e, também, a entrada de glicose para a musculatura esquelética (27).

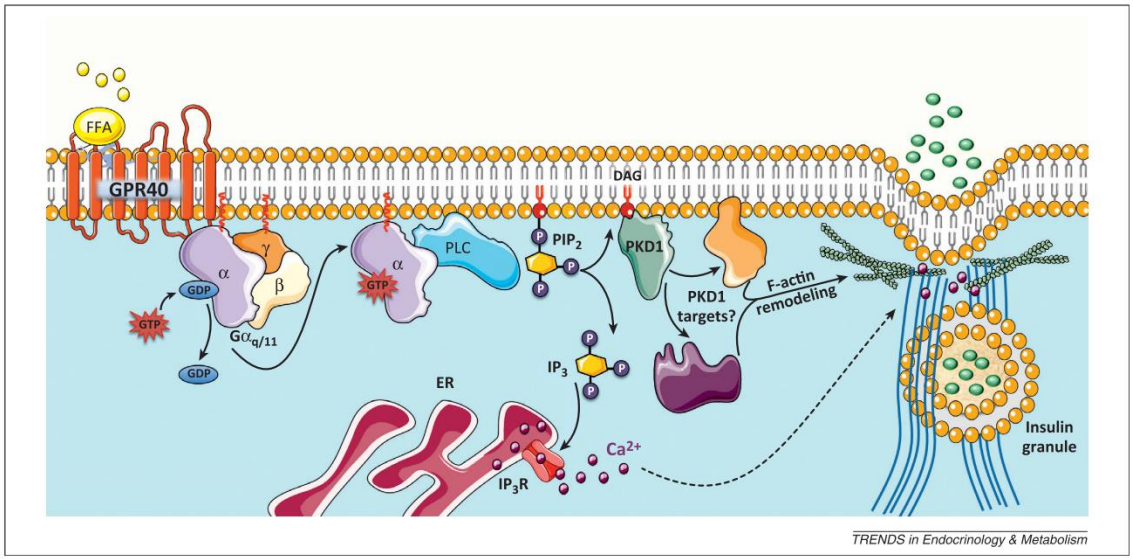

Figura 6 Sinalização intracelular após ativação do FFA1, levando à secreção de insulina.
Retirado de Mancini & Poitout (25). Licença para uso: 4610981175204.

No pâncreas, a liberação de insulina dependente de FFA1 ocorre por ativação desse receptor nas células β-pancreáticas, via proteína quinase C(PKC)/inositol trifosfato (IP_3), levando à elevação de Ca^{++} e , por consequência, à liberação de insulina (Figura 6) (28,29). Porém, sabe-se que a falta desse receptor não protege os animais de desenvolverem diabetes e/ou síndrome metabólica (30,31). Em relação ao papel desse receptor na secreção de insulina independente de glicose, pode-se dizer é uma ação contraditória, podendo ser benéfica ou maléfica para o organismo, podendo gerar um quadro de hiperinsulinemia (32).

O FFA1 tem sido amplamente investigado para o tratamento de diabetes mellitus 2, incluindo testes clínicos de fase II com o agonista sintético seletivo TAK-875 (33). Nesse caso, os ensaios foram descontinuados em função do desenvolvimento de hepatotoxicidade nos pacientes tratados com o agonista (34).

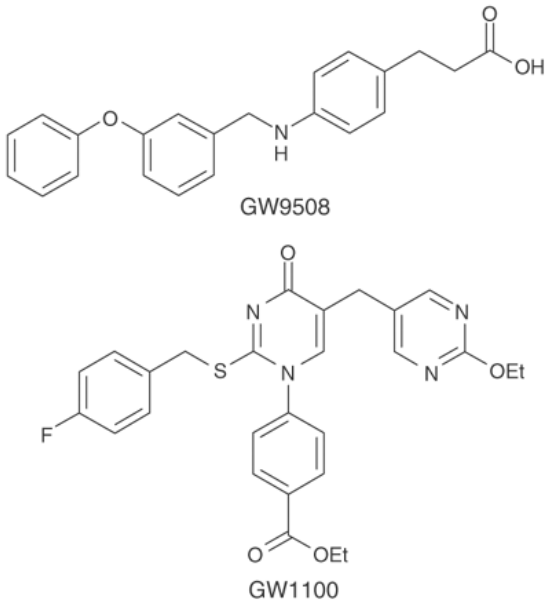

Figura 7 Estruturas químicas do agonista dual FFA1/FFA4, GW9508, e do antagonista seletivo para FFA1, GW1100. Retirado de Briscoe et al (30). Licença para uso: 4650320296741

Entretanto, utilizando tanto os ácidos graxos poli-insaturados previamente citados, como outras moléculas sintéticas, como o GW1100 e o GW9508 (Figura 7), há uma grande quantidade de evidências mais recentes demonstrando a relevância desse receptor em processos metabólicos e ao nível do SNC (28,35–39). Vale ressaltar que, embora o GW9508 seja considerado um agonista não seletivo para o FFA1, ele possui 100x mais afinidade por esse receptor do que para o FFA4 (29). Na Tabela 2, encontra-se os ligantes sintéticos do FFA1.

Tabela 2 Ligantes sintéticos do FFA1 e suas especificidades.
Adaptada de Alquier & Poitout (32)

Ligante Sintético	Tipo de ligante	Especificidade
GW9508	Agonista	Agonista não seletivo*
NCG21	Agonista	Agonista não seletivo*
TUG-891	Agonista	Agonista não seletivo[#]
TAK-875	Agonista	Agonista seletivo
P11187	Agonista	Agonista seletivo

LY292470	Agonista	Agonista seletivo[$]
SHR0534	Agonista	Agonista seletivo
GW1100	Antagonistas	Antagonista seletivo

*Também atua sobre o FFA4; [#]Seletivo para o FFA4 em humanos, mas se liga em ambos os receptores em camundongos; [$]Possui afinidade pelos PPARs.

De maneira interessante, no SNC, a ativação do FFA1 parece ser relevante para o controle da dor, uma vez que sua estimulação, tanto na medula espinhal como no cérebro, leva à liberação de β-endorfina e IL-10 (36,40–42). Além disso, o FFA1 está expresso em diferentes regiões cerebrais como: córtex, ponte, medula oblongata, subtância nigra, amígdala, giro dentado do hipotálamo, locus cereleus e na hipófise (43,44). Mais recentemente, em um modelo de estresse combinado com dor pós-cirúrgica, o receptor FFA1 participa de forma ativa na dor, mas não nos efeitos emocionais da dor relacionada ao estresse (45). De maneira interessante, o FFA1 não está apenas envolvido no controle da dor, quando se pensa em SNC (46). Observou-se que em animais *knockout* para o receptor FFA1, houve um aumento do comportamento depressivo e ansioso, além dos níveis de noradrenalina no hipocampo, hipotálamo, mesencéfalo e bulbo(47). Essa mesma deleção gerou alterações emocionais em relação à prole em camundongos fêmeas, como negligência (48). Além disso, a ativação do FFA1 se mostrou um promissor alvo terapêutico para desordens neurológicas como epilepsia e esclerose múltipla (46,49).

De maneira notável, observou-se que em animais eutróficos, o FFA1 está localizado em neurônios hipotalâmicos que expressam tanto neuropeptídeo Y (NPY) como propriomelanocortina (POMC), indicando sua participação na regulação da homeostase energética. Ainda, o tratamento i.c.v. com o agonista GW9508 reduziu a inflamação hipotalâmica de animais obesos (50). Também, a ativação do FFA1 por AgoPAMs (agonistas que agem como moduladores alostéricos) estimulou a secreção do

peptídeo semelhante ao glucagon-1 (GLP-1) em animais obesos, levando à um decréscimo de peso corporal (51). Além disso, em um modelo de diabetes e obesidade associado a déficits de memória e aprendizado, há uma diminuição na sinalização do FFA1 no hipotálamo e córtex, e esse aspecto foi revertido pelo tratamento com DHA e com o agonista sintético GW9508 (52). De forma interessante, em pacientes com síndrome metabólica e periodontite, a expressão de FFA1 se mostrou exacerbada nos tecidos periodontais desses pacientes, demonstrando que este receptor é um alvo terapêutico em potencial (53).

Mais recentemente, a expressão do FFA1 tem sido avaliada em células cancerígenas, como um potencial alvo farmacológico. Observou-se que, em pacientes com câncer colorretal, a alta expressão deste receptor no tumor, juntamente com altos níveis de triglicerídeos, está associada a uma menor sobrevida global (54). Em um estudo pré-clínico, uma dieta rica em ômega-3 diminuiu a incidência de tumores na região colorretal. Além disso, em células de câncer colorretal, o tratamento com EPA e DHA aumentou a morte celular por apoptose. Os efeitos *in vitro* e *in vivo* se devem à ativação do receptor FFA1 (55). Por outro lado, a expressão de FFA1 está aumentada em tecidos de câncer de próstata, o qual foi estimulado pelo tratamento com ácido oleico, demonstrando que o FFA1 é um potencial biomarcador e um alvo terapêutico para esse tipo de câncer (56).

1.2.2 Receptor de ácidos graxos livre 4 (FFA4)

Em 2005, o FFA4, conhecido anteriormente como GPR120, foi deorfanizado; foi observado que quando ativado, estimulava a secreção de GLP-1 *in vitro* e *in vivo* (21). Esse receptor, em humanos, existe em duas isoformas: longa e curta. A longa contém um segmento residual de 16 aminoácidos inserido na terceira alça intracelular, que desassocio receptor da proteína G. Já a curta, na sua totalidade, contém 361 aminoácidos e possui um peso molecular de 42 kDa (Figura 8) (57).

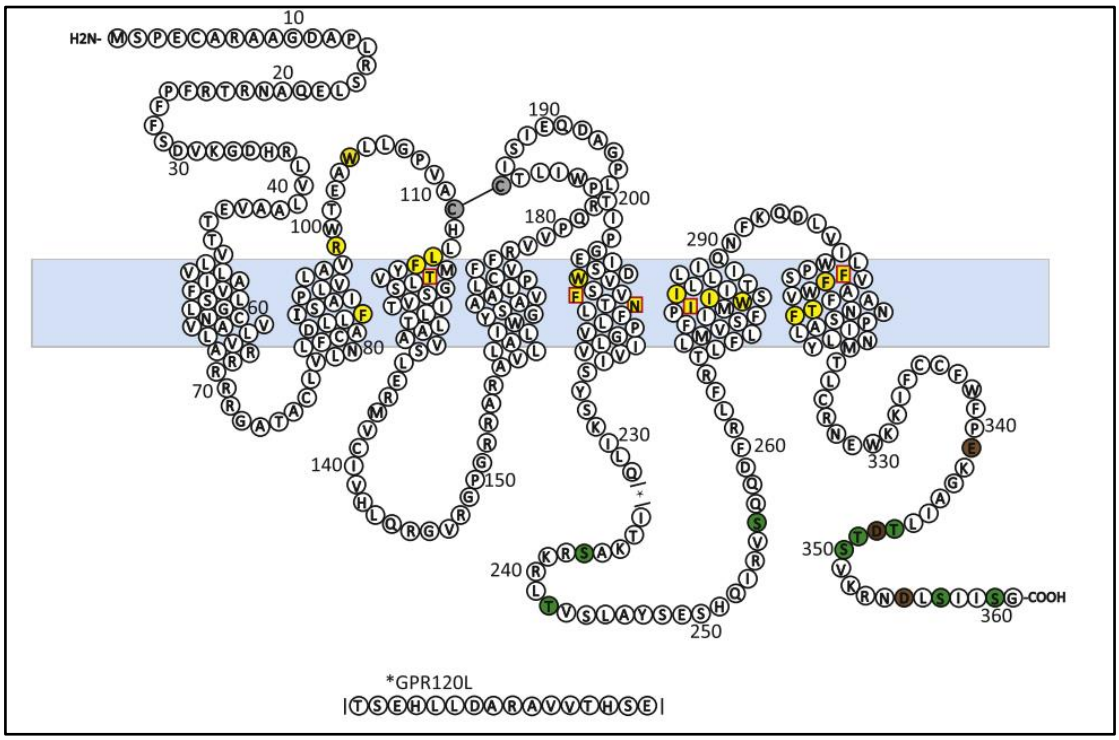

Figura 8 Estrutura do FFA4 (longa e curta). Retirado de Ulven & Christiansen (59). Licença para uso: 610990466424

O FFA4, como o FFA1, também é estimulado por diferentes ácidos graxos de cadeia longa, como o ALA, ácido mirístico e o ácido oleico (22). Demais ligantes estão descritos na Tabela 1(24). Para que o estudo desse receptor fosse mais preciso, uma vez que os ácidos graxos livres são farmacologicamente promíscuos, uma série de moléculas

sintéticas foram criadas. Abaixo, pode-se observar a Tabela 3 que descreve os principais ligantes e sua especificidade.

Tabela 3 Ligantes sintéticos do FFA4 e suas especificidades.
Adaptado de Alquier & Poitout (32)

Ligante Sintético	Tipo de ligante	Especificidade
GW9508	Agonista	Agonista não seletivo*
16:4 (n-3)	Agonista	Agonista não seletivo*
NCG21	Agonista	Agonista seletivo
TUG-891	Agonista	Agonista seletivo[#]
KDT501	Agonista	Agonista seletivo[$$]
AH7614	Antagonistas	Antagonista seletivo

*Também atua sobre o FFA4; [#]Seletivo para o FFA4 em humanos, mas se liga em ambos os receptores em camundongos; [$$]Agonista para o FFA4 com atividade em outros GPCRs e PPAR-γ

É possível afirmar que esse receptor tem um papel importante para a regulação do metabolismo energético, uma vez que é altamente expresso em locais como o tecido adiposo, intestino, pâncreas, fígado, papilas gustativas, hipotálamo e nos macrófagos (58). Porém, ambas as isoformas ativam β-arrestina-2, levando à internalização do receptor. A isoforma curta parece ser a responsável pela secreção hormonal e a translocação do transportador de glicose tipo 4 (GLUT4) via proteína $G_{q/11}$ e pela mobilização de cálcio (59). Em função dessa regulação hormonal, esse receptor tem sido amplamente investigado como um alvo terapêutico para diabetes tipo 2, resistência à insulina e obesidade (58–60). Animais *knockout* para o receptor FFA4 desenvolvem uma obesidade mais grave do que animais *wild-type*, quando ambos ingerem a mesma dieta rica em gordura. Em humanos, além da expressão do receptor ser menor em indivíduos obesos, quando comparados com indivíduos eutróficos, há uma mutação no gene do receptor, levando a uma ineficiência da sinalização do FFA4 nesses indivíduos (61,62).

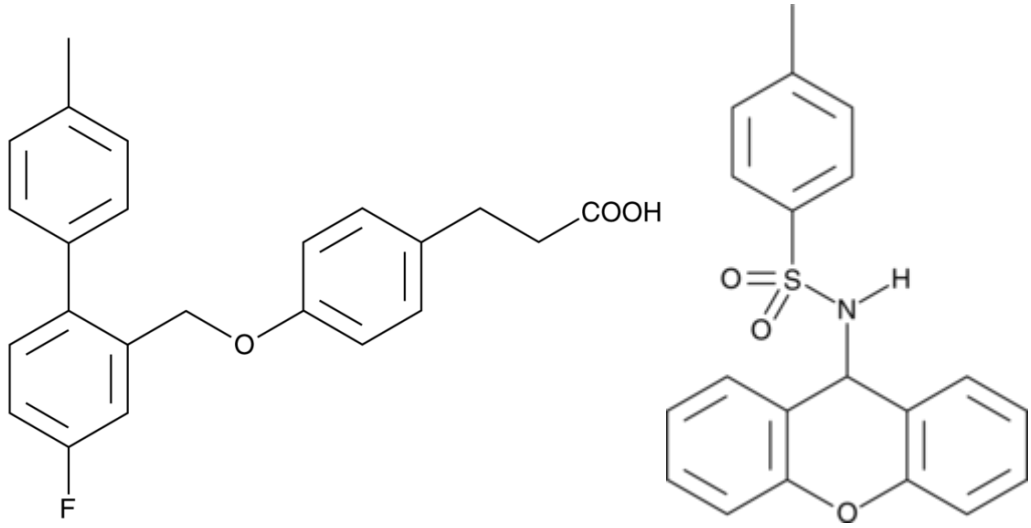

Figura 9 Estruturas químicas do agonista para FFA4, TUG891 (esquerda), e o antagonista seletivo para o FFA4, AH7614 (direita). Retirado de Alexander et al (22). Produzido por *International Union of Basic and Clinical Pharmacology* (IUPHAR) – Acesso livre.

Em relação à inflamação, o recrutamento de β-arrestina-2 parece ser o principal mecanismo nos macrófagos, uma vez que a internalização do receptor leva à inibição de quinase ativada pelo fator transformador de crescimento-1 (Tak-1), uma proteína quinase ativada por mitógenos (MAPK) que permite a translocação do fator nuclear κB (NF-κB) para o interior do núcleo destas células, gerando uma resposta pró-inflamatória (Figura 8) (59,63).

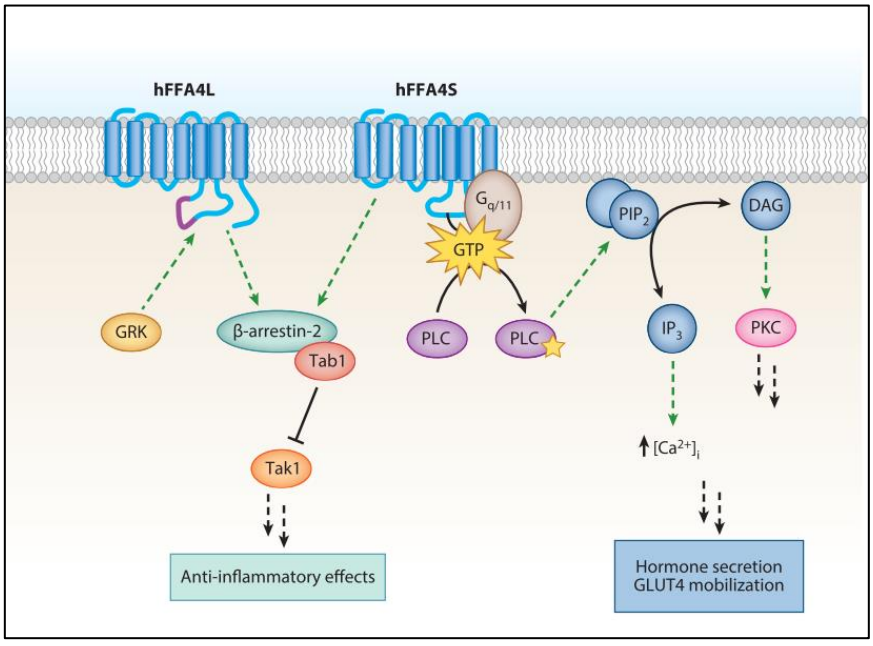

Figura 10 Sinalização intracelular das duas isoformas do FFA1 humano. Retirado de Ulven & Christiansen (59). Licença para uso: 4610990874650.

De maneira interessante, o papel do FFA4 no sistema nervoso central é menos investigado do que o papel do FFA1 nesse tecido. Porém, já existem evidências que esse receptor também está expresso no cérebro, como no hipotálamo e na hipófise. Em camundongos, foi demonstrado que a ativação central do FFA4 levou à diminuição da ingesta alimentar, do comportamento de recompensa e do comportamento ansioso (64). Por outro lado, Dragano *et al*., demonstrou que o FFA4 está expresso, principalmente, na microglia do hipotálamo (50). Além, disso, o FFA4 exerce um papel importante sobre os efeitos de transformação do tecido adiposo branco em tecido adiposo marrom, o qual se viu sua participação em camundongos *wild-type* sem indução de obesidade e em animais expostos ao frio (65,66).

Já, em relação ao câncer, esse receptor se mostra presente em diferentes linhagens celulares como câncer de pulmão, câncer de mama, melanoma e câncer ósseo (67). Além disso, a ativação de FFA1 em células de câncer de mama promove metástase, via PI3K/proteína quinase B(Akt)/NF-κB (68). Por outro lado, em células de carcinoma de pulmão de Lewis (LLC), a ativação do FFA4 regulou negativamente a motilidade celular demonstrando que, possivelmente, a ativação deste receptor nesta linhagem está associada à diminuição do surgimento de metástases (69).

1.3 Síndrome da anorexia-caquexia

A caquexia é uma síndrome multifatorial definida pela perda de massa muscular (com ou sem perda de gordura), que não é revertida plenamente por um suporte nutricional convencional, levando a uma perda funcional progressiva (70). Porém, ainda

é muito controversa a definição desta síndrome, uma vez que pacientes caquéticos diferem em composição corporal e perda de peso (71). Além disso, pacientes que apresentam alto grau de caquexia sofrem mais com os efeitos tóxicos da quimioterapia, quando comparados aos pacientes oncológicos sem a síndrome. Porém, os mecanismos dessa condição ainda não foram elucidados (72,73). Ademais, presença da massa tumoral gera uma gama de fatores que podem influenciar diretamente o estado nutricional do paciente oncológico, como mostra a Figura 9.

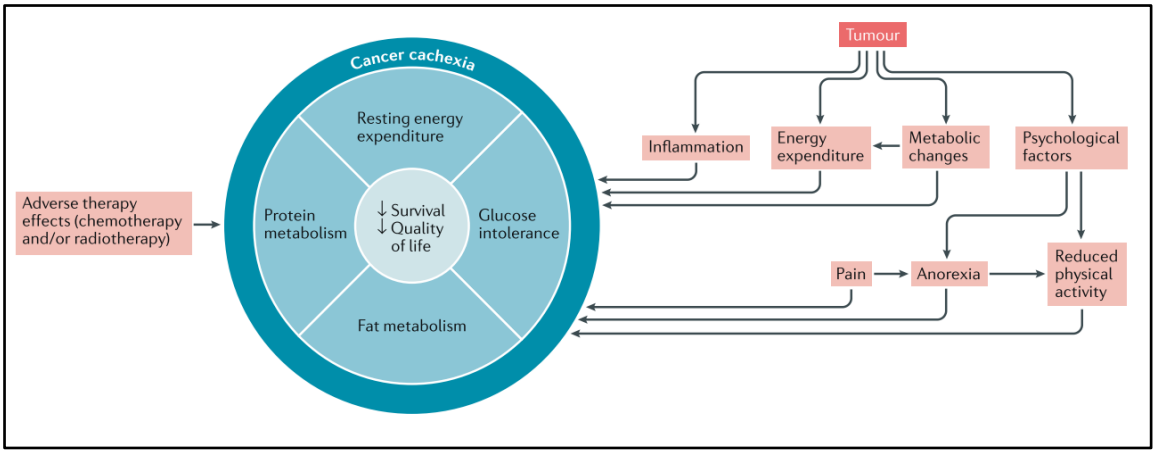

Figura 11 Fatores que contribuem para o desenvolvimento do quadro caquético. Retirado de Argilés et al. (75). Licença para uso: 4611000252771

Geralmente, a caquexia afeta aproximadamente 50 % dos pacientes oncológicos, sendo que desses, em torno de 20 % vão a óbito devido à síndrome (74,75). A severidade do quadro caquético se deve, principalmente, ao tipo de tumor: 80 % dos pacientes com câncer gástrico ou pancreático desenvolvem caquexia, 50 % dos pacientes com câncer de pulmão, próstata ou cólon e, 40 % dos pacientes leucêmicos ou com câncer de mama também apresentam essa síndrome (75).

Além de afetar pacientes oncológicos, a caquexia também pode se desenvolver em outras patologias, como doenças renais crônicas, doenças cardiovasculares, doença

pulmonar obstrutiva crônica, infecções, trauma, doenças neurológicas, hepatopatias, entre outras (76).

O CASCO (*cachexia score*) surgiu como uma forma de detectar e classificar estes pacientes por meio de uma equação. Essa equação é composta por: perda/composição de peso corporal (BWC) + inflamação/desordens metabólicas/imunossupressão (IMD) + performance física (PHP) + anorexia (ANO) + qualidade de vida (QOL) (77). Entretanto, como os próprios autores desse escore concluem, essa é uma ferramenta completa, porém, de difícil aplicação na rotina hospitalar. De qualquer maneira, a Sociedade Europeia de Nutrição Enteral e Parenteral (ESPEN) recomenda que todos os pacientes oncológicos devem ser classificados como risco nutricional, independente do peso corporal e do percentual de peso perdido, além de aconselhar que outros fatores sejam triados pelo nutricionista como presença de anorexia, composição corporal, marcadores inflamatórios, gasto energético basal e funcionalidade. Também, as duas diretrizes da ESPEN voltados para o manejo nutricional em pacientes oncológicos incentivam o uso intervenções nutricionais que sejam voltadas para a diminuição do quadro inflamatório e hipermetabólico (78,79).

Os principais parâmetros que devem ser considerados para detecção da síndrome da anorexia-caquexia são: perda grave de peso (>10% nos últimos 6 meses ou >5% no último mês), índice de massa corporal baixo (IMC < 18 kg/m²), Avaliação Subjetiva Global < C e albumina sérica menor que 30 g/L (80–82). Vale ressaltar que, embora o IMC ainda seja utilizado como uma forma de detecção para baixo peso é uma medida que pode não se mostrar relevante, uma vez que pacientes oncológicos caquéticos podem ser obesos conforme peso corporal e IMC (83). O Escore de Prognóstico de Glasgow modificado, que é uma combinação entre a proteína C-reativa e a albumina sérica, é uma importante ferramenta para correlacionar risco nutricional, perda de peso com a

diminuição da resposta ao tratamento (84). Também, uma alta razão neutrófilo-linfócito também é considerada um interessante marcador para perda de peso e diminuição da sobrevida do paciente (85). Além de tudo, o percentual de perda de peso, juntamente com o IMC, pode ser utilizado como um preditor para sobrevida do paciente (86)

Além dessas avaliações, o teste de força da preensão palmar também é empregado: a perda de força do paciente está diretamente relacionada à perda muscular e à desnutrição (87). Para uma análise mais precisa de todos os compartimentos teciduais, podem ser utilizados exames de imagem, como PET/CT e ressonância magnética (88). Outra forma de quantificar os diferentes tecidos é através do teste de bioimpedância, incluindo a análise do ângulo de fase (89). O método mais utilizado, atualmente, nos estudos clínicos, é a densitometria por dupla emissão de raios-X (DEXA). Porém, a tomografia computadorizada ainda é a primeira escolha em função de limitações, como aumento dos custos do estudo (71).

Fearon *et al.* (2011), além de conceituarem esta síndrome, definiram os estágios de progressão da caquexia (Figura 10) (70). A primeira denomina-se **pré-caquexia**, onde o paciente apresenta alterações metabólicas (anorexia e intolerância à glicose), além de perda de peso maior ou igual a 5 % do peso inicial. O risco de piora do quadro varia de acordo com a resposta do paciente ao tratamento antitumoral, com o tipo de tumor e com a baixa ingestão alimentar. Os pacientes que alcançam a fase dois, considerada fase de **caquexia**, devem apresentar perda de peso maior do que 5 % durante os últimos 6 meses, ou IMC menor que 20 kg/m^2 ou 2 % de perda de peso corporal associada à sarcopenia. A última fase é chamada de **caquexia refratária**, onde o paciente apresenta grave perda de peso, em consequência de um câncer pré-terminal ou não responsivo ao tratamento. Também é caracterizada por um status de desempenho baixo e uma expectativa de vida menor de três meses, sendo mais facilmente detectável (70). O reconhecimento da

existência de três fases em que a síndrome obrigatoriamente se desenvolve pode levar à confirmação da caquexia, em conjunto com o diagnóstico da doença primária como forma de prevenção (90).

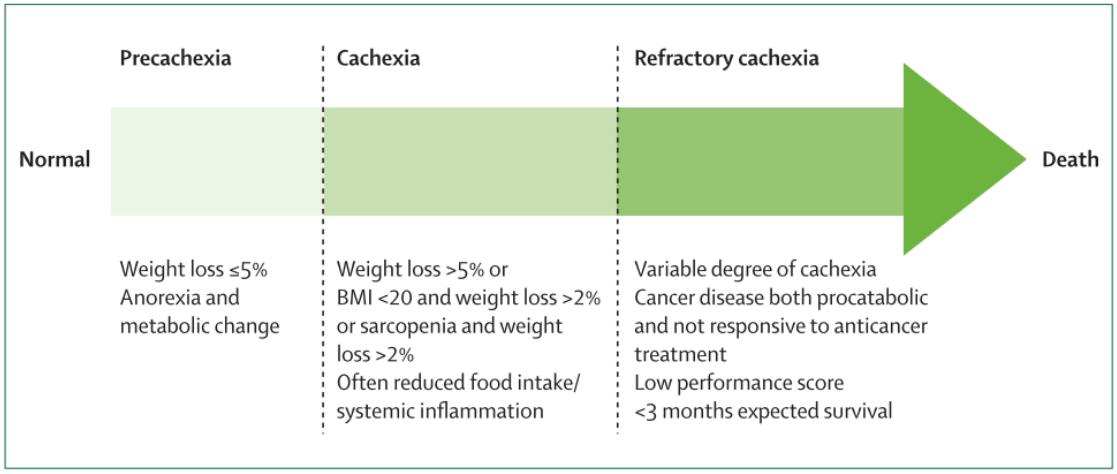

Figura 12 Estágios de desenvolvimento da caquexia relacionada ao câncer. Retirada de Fearon et al (71). Licença para uso: 4611000159842

Parte dos diagnósticos de câncer são realizados em indivíduos obesos (91). Como estes pacientes não apresentam o decréscimo de IMC, nem a perda de peso corpóreo necessária para serem classificados como caquéticos, o quadro pode passar despercebido e deixar de ser tratado. Nestes pacientes, a caquexia pode ser detectada por imagem – quando é possível observar uma diminuição da massa muscular e aumento do tecido adiposo (86,91).

1.3.1 Anorexia

Pacientes que desenvolvem síndrome de anorexia-caquexia, normalmente, apresentam decréscimo do apetite e alterações no paladar, ocasionados principalmente pela inflamação hipotalâmica, o que potencializa a perda de gordura corporal e atrofia muscular (92). Mecanismos centrais e periféricos podem levar ao desenvolvimento da anorexia relacionada ao câncer. As causas periféricas podem ser o local do tumor

(tumores gastrointestinais, por exemplo), substâncias produzidas pelo tumor que podem alterar a ingestão alimentar, bem como, substâncias que podem alterar os nutrientes e o paladar. Além disso, o tratamento oncológico pode contribuir para a anorexia, pois é capaz de alterar o paladar, causar náuseas, vômitos, mucosite e dores abdominais. Por outro lado, os mecanismos centrais da anorexia associada ao câncer estão relacionados à inflamação hipotalâmica, consequentemente, ao desbalanço do sistema melanocortina, e às alterações de neurotransmissores (93–95).

O sistema melanocortina (Figura 11) está localizado no núcleo arqueado do hipotálamo, adjacente ao 3º ventrículo. A barreira hematoencefálica desta região é relativamente permeável, o que possibilita a passagem de citocinas pró-inflamatórias, entre outras substâncias. O sistema melanocortina possui dois tipos de neurônios, os quais funcionam de maneiras opostas em relação à regulação do apetite (96,97). A primeira classe de neurônios é de origem anorexígena e expressa POMC e o transcrito regulado pela cocaína e anfetamina (CART). O POMC, quando clivado, se transforma em hormônio estimulante de α-melanócitos (α-MSH). Neurônios que expressam POMC/CART realizam sinapse com neurônios de segunda ordem em diversas áreas do cérebro e do tronco cerebral. Uma vez que o α-MSH é liberado na fenda sináptica desses neurônios, ele se liga aos receptores de melanocortina 3 e 4 (MC3R e MC4R), que provocam diminuição de apetite, redução de massa muscular e aumento da taxa metabólica basal. Os neurônios da segunda classe são classificados como orexígenos e expressam o NPY e a proteína relacionada à Agouti (AgRP). A AgRP é um agonista inverso de MC4R; logo, quando se liga a esse receptor, produz um aumento de apetite e diminuição da taxa metabólica basal (96,98,99).

A leptina é um hormônio anorexígeno produzido pelo tecido adiposo branco e pelo sistema digestório (100). Sua principal função é inibir a fome; o desenvolvimento de

resistência à leptina representa uma das principais causas de obesidade mórbida (101). A leptina é capaz de ultrapassar a barreira hematoencefálica e ativar seu receptor (Ob-Rb) no SNC nos neurônios POMC, estimulando a liberação de α-MSH (99). A relação entre a leptina e a caquexia induzida pelo câncer ainda é controversa, pois há um decréscimo de leptina circulante em pacientes caquéticos e que mesmo assim apresentam falta de apetite (102). Essa diminuição de leptina está relacionada à resistência à insulina e ao aumento de fator de necrose tumoral (TNF) (103). Por outro lado, uma metanálise verificou que altos níveis séricos e teciduais de leptina estão relacionados à progressão do câncer de pulmão, em uma população chinesa; porém, não haveria relação entre altos níveis de leptina e desenvolvimento de caquexia (104).

A grelina é um hormônio orexígeno produzido pelas células endoteliais do estômago quando este se encontra vazio (105). As ações da grelina são mediadas pelo receptor secretagogo do hormônio do crescimento (GSH-R), que apresenta alta expressão no hipotálamo, principalmente nos neurônios NPY/AgRP (106). Quando a grelina ultrapassa a barreira hematoencefálica, se liga ao seu receptor, estimulando a liberação de AgRP na fenda sináptica, fazendo com que haja o estímulo da fome (98,107). Além de estimular o apetite, a grelina possui diferentes funções no organismo: em outras regiões do cérebro, participa do processo de aprendizado, memória e no sistema de recompensa; em outros órgãos pode diminuir a pressão arterial, promover vasodilatação e motilidade gástrica e intestinal (108). Nos pacientes caquéticos, pode ser observada uma hipergrelinemia, um mecanismo compensatório, porém falho, havendo um decréscimo da sinalização de grelina no hipotálamo (109). Todavia, pacientes oncológicos anoréticos mostraram aumento da ingestão alimentar após infusões de grelina, demonstrando um efeito benéfico deste peptídeo para essa população em questão (110).

Embora o sistema melanocortina seja regulado, principalmente, por leptina e por grelina, os níveis de serotonina no hipotálamo também são de extrema importância para a homeostase da região (111). A inflamação hipotalâmica induz aumento nos níveis de serotonina no hipotálamo, estimulando a via anorexígena da POMC e inibindo a secreção de NPY, indicando que a serotonina tem um papel importante no desenvolvimento da anorexia associada ao câncer (112,113). Já foi demonstrado que animais que desenvolveram anorexia associada ao câncer apresentam um aumento de expressão de receptor 5-HT1_B no hipotálamo, enquanto os receptores para NPY tiveram sua expressão reduzida (114). Além disso, o mesmo grupo de pesquisadores demonstrou que a ressecção tumoral reverteu os parâmetros associados à serotonina, no que se refere à anorexia induzida pelo câncer (115). Ademais, o triptofano, precursor da serotonina, participa de forma ativa no desenvolvimento da anorexia associada ao câncer, gerando um acúmulo de serotonina no hipotálamo (116).

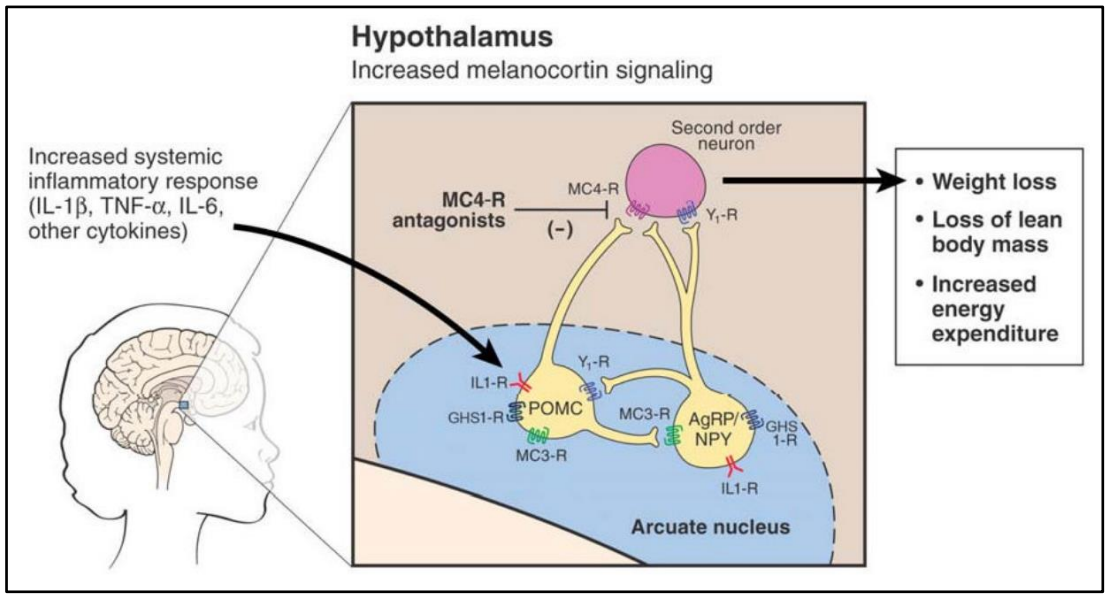

Figura 13 Sistema melanocortina na caquexia associada ao câncer. Retirado de DeBoer (98). Licença de uso: 4610991193302

A inflamação hipotalâmica (Figura 12) é um importante mecanismo da síndrome da anorexia-caquexia (112). Um fator crucial para o desenvolvimento da inflamação hipotalâmica é a neuroinflamação ocasionada pelo estresse crônico originário do tumor, levando a um aumento da ativação microglial (111). Notavelmente, observou-se que pacientes oncológicos anoréticos apresentam uma menor atividade do hipotálamo, tanto quando se alimentam, como quando apenas visualizam um alimento, demonstrando que a região do hipotálamo é afetada pela doença (95,117).

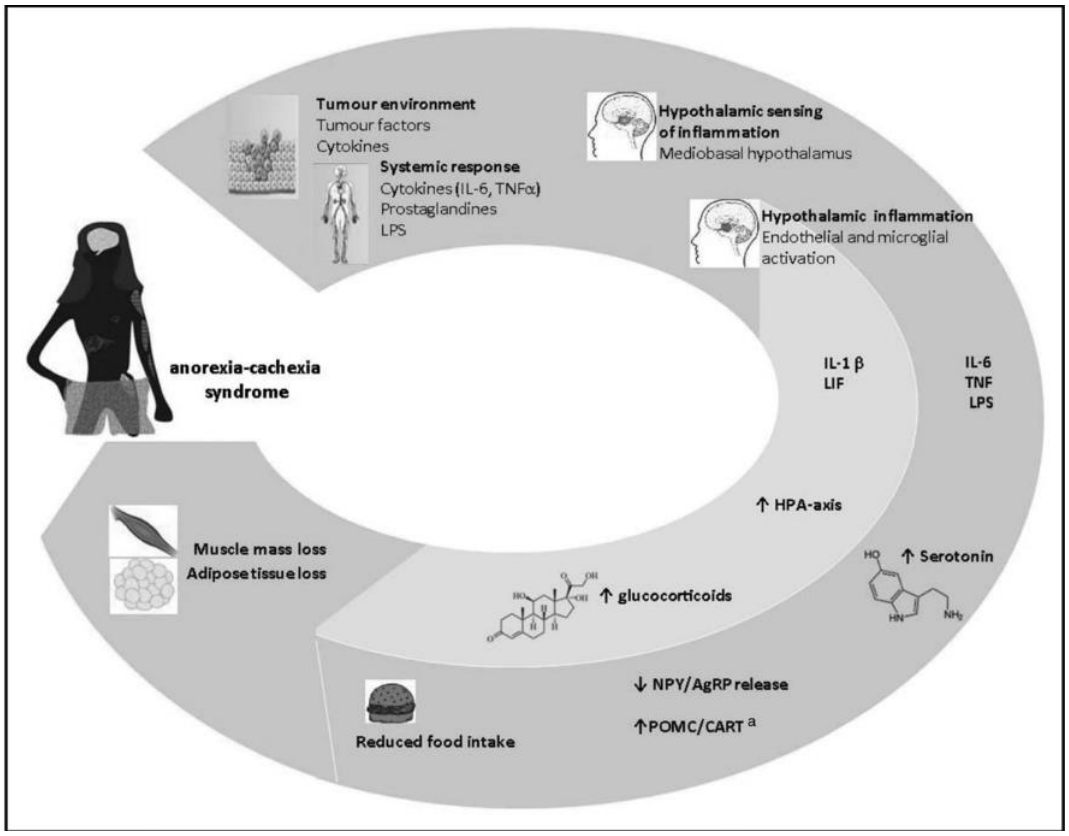

Figure 14 Inflamação hipotalâmica na caquexia associada ao câncer. Retirado de Norren et al (94). Licença para uso: 4610991485368

Além disso, experimentos em animais demonstraram que a administração i.c.v de TNF gerou um redução de peptídeos secretados pelo sistema melanocortina, levando a um aumento do metabolismo e diminuição da ingestão alimentar em ratos (118). Também, a interleucina (IL)-1β, quando administrada pela mesma via, altera a

homeostase metabólica em animais (119). Já, em pacientes com câncer avançado, TNF, IL-1β, proteína quimioatrativa de monócitos (MCP)-1, fator de crescimento transformador (TGF)-β, entre outros mediadores, estão associadas à inapetência relacionada ao câncer (120). De maneira interessante, tanto TNF como IL-1β possuem papéis importantes na modulação neuroendócrina na caquexia, uma vez que ambas são permeáveis à barreira hematoencefálica e alcançam o núcleo arqueado do hipotálamo (121). No que se refere à IL-1 β, esta citocina pode tanto ativar os neurônios POMC, como inibir os neurônios NPY/AgRP, levando à uma inibição do apetite, aumento do gasto energético basal e maior gasto muscular esquelético (96,122). Por outro lado, a administração i.c.v. de TNF leva ao quadro anoréxico e ao hipermetabolismo pela via JAK/STAT, semelhante ao efeito da leptina no hipotálamo (123).

1.3.2 Atrofia muscular

A atrofia muscular parece ser a manifestação clínica da caquexia mais relevante e, está diretamente associada a um prognóstico ruim (124). A reversão dessa condição está relacionada à melhora do quadro geral do paciente, sendo que a prevenção da perda de massa muscular é benéfica para a sobrevida (125).

O principal mecanismo de atrofia muscular na caquexia é a ativação do sistema ubiquitina-proteassoma. A ubiquitinação envolve uma série de reações mediadas por três classes de proteínas: as enzimas ativadoras de ubiquitina (E1s), as enzimas conjugadoras de ubiquitina (E2s) e as ligases proteína-ubiquitina (E3s). Esse processo inicia com a ativação da ubiquitina, dependente de adenosina trifosfato (ATP), por uma E1. Essa ubiquitina ativada é transferida para uma E2 e, então, transferida para a E3, via um substrato para uma E3 ligase. Posteriormente, a proteína ligada à ubiquitina é transferida para o proteassoma 26S, onde é degradada. As enzimas E3 ligases Murf1 e Atrogina-

1/MAFbx estão super-expressas em quadros de caquexia e sua importância na atrofia muscular se deve ao fato de que essas enzimas se ligam ao filamento mais espesso do sarcômero (126,127).

Uma das vias de extrema importância para o desenvolvimento da atrofia muscular é a do fator de crescimento semelhante à insulina -1 (IGF-1)/insulina-PI3K-AKT. Em situações normais, onde há produção de IGF-1 e insulina, esta via é ativada e induz a hipertrofia e o aumento de força muscular. A produção muscular, por essa via, se deve à ligação do IGF-1/insulina ao seu receptor, que ativa a PI3K/AKT/mTOR, que por sua vez inibe o fator de transcrição FoxO, aumentando a síntese proteica e inibindo a degradação. Em quadros patológicos, em que há ausência de insulina ou resistência à mesma, há uma inversão dessa via, onde ocorre inibição da via da PI3K/AKT, ativação do fator de transcrição FoxO, cuja expressão leva à produção de atrogina-1(126,128,129). Vale ressaltar a importância da resistência à insulina nos pacientes oncológicos, já que esta é uma das principais alterações metabólicas presentes na caquexia induzida pelo câncer, observada em diversos tipos de tumores, representando um dos principais mecanismos de atrofia muscular (130,131).

Há outras vias que também levam à ativação do sistema ubiquitina-proteassoma, sendo estimuladas por um mesmo grupo de moléculas. Tanto a via do fator nuclear-κB (NF-κB), como a via da MAPK p38, são estimuladas por citocinas pró-inflamatórias produzidas pelo tumor ou, por células normais, na tentativa de combater o tumor (126,128,132). Deve-se ressaltar que a IL-6 e o TNF agem em conjunto para que a atrofia muscular aconteça (133). Altas quantidades de TNF parecem estar relacionadas diretamente à resistência à insulina, uma das principais características da caquexia. O TNF, quando se liga ao seu receptor, por estímulos intracelulares, impede a sinalização da insulina, provocando uma ineficiência dos receptores de insulina quando ativados pelo

seu ligante (134). Já, a interleucina-1β (IL-1β), também exerce papel similar de ativação das mesmas vias, mas com menor importância (97,128).

Além das citocinas citadas acima, o *TNF-like Weak Inducer of Apoptosis* (TWEAK) é um importante alvo para o tratamento da caquexia pois, em situações patológicas, sua expressão está aumentada (135,136). O TWEAK faz parte da família do TNF e possui diferentes mecanismos: em baixas concentrações, o TWEAK possui efeito anabólico, levando à miogênese, e em altas concentrações, possui efeito catabólico, gerando atrofia muscular. A atrofia muscular desencadeada por TWEAK se deve à ativação do segundo mensageiro TRAF6 que, por sua vez, ativa NF-κB e MURF-1 (137). Os principais mecanismos de atrofia estão ilustrados na Figura 13.

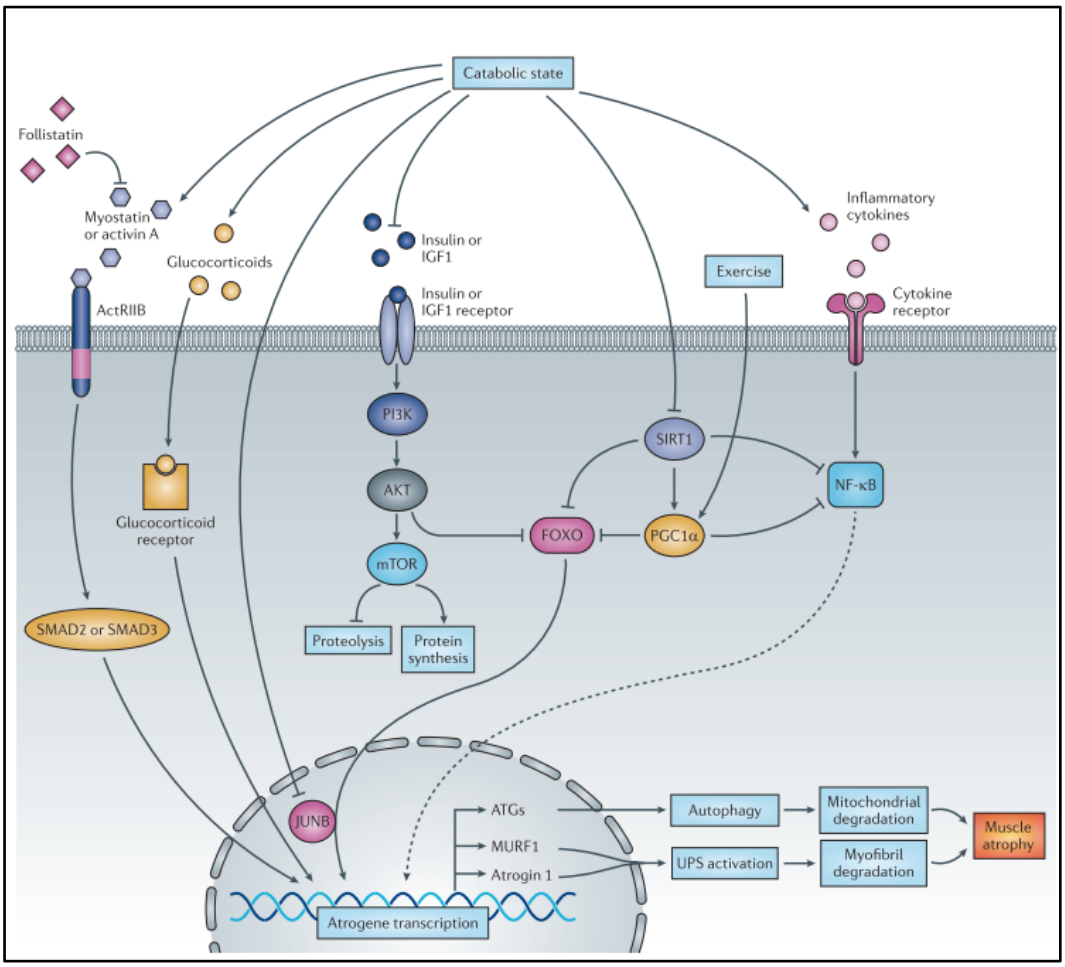

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Figura 15 Mecanismos de atrofia muscular. Retirado de Cohen et al (128). Licença para uso: 4610991280834

A miostatina é um fator de crescimento produzido pelos miócitos com objetivo de inibir a miogênese, numa forma de regulação autócrina. A miostatina faz parte da superfamília TGF-β, juntamente com a activina (138). Ambas as proteínas estão super-expressas no câncer, que, se ligando ao receptor B Activina II (ActIIb) estimula a produção dos fatores de transcrição SMAD2 e SMAD3 (139). O aumento de miostatina está relacionado com a resistência à insulina; porém, esse mecanismo ainda é desconhecido. O que se sabe é que a inibição da miostatina e de outras proteínas da super-família TGF-β pode estar relacionada à ativação da via PI3K/AKT/mTOR, estimulando a hipertrofia muscular, auxiliando na reversão do quadro caquético (126,128,140)

1.3.3 Perda de gordura e aumento do metabolismo energético

A perda de gordura corporal na caquexia associada ao câncer está relacionada ao decréscimo na ingestão alimentar (141). Porém, a anorexia *per se* não é capaz de provocar uma diminuição tão expressiva de gordura, como é observado na caquexia. Logo, pode-se propor que esteja associada também ao aumento do metabolismo energético (141,142).

O tumor necessita de nutrientes para o seu desenvolvimento e progressão; portanto, ambos: atrofia muscular e gasto de gordura corporal são importantes para a manutenção e o desenvolvimento do tumor (143). A principal fonte de energia para os tumores sólidos é a glicose, que é transformada em lactato, ao invés de gás carbônico. Isso ocorre porque não há oxigênio suficiente para que ocorra o ciclo de Krebs e o restante do metabolismo aeróbio. O lactato é transferido para o fígado, que utiliza a cadeia carbônica para a gliconeogênese. Outros substratos também contribuem para a gliconeogênese aumentada nessa situação (142–144).

Alguns fatores liberados pelo tumor, como a IL-6, o TNF, a proteína relacionada ao hormônio da paratireoide (PTHrP), o fator mobilizante de lipídeos (LMF) e a zinco α2-glicoproteína (ZAG; liberada pelo tecido adiposo), e o fator inibidor de leucemia (LIF) que estimulam a ativação do sistema β-adrenérgico, que induz o processo de lipólise, que consiste em uma cascata de reações mediadas por três enzimas (141,145–149). Primeiramente, a lipase do triglicerídeo do tecido adiposo (ATGL) quebra o triaciglicerol (TAG) em diacilglicerol (DAG). Depois, a lipase sensível ao hormônio (HSL) transforma o DAG em monoacilglicerol (MG) e, finalmente, a MG lipase o transforma o MG, separando o glicerol da cadeia de ácido graxo (150).

O tecido adiposo branco é constituído, em sua maioria, por adipócitos brancos, os quais são compostos por uma grande gotícula de gordura e um pequeno núcleo deslocado, com pouca quantidade de mitocôndrias. Em contrapartida, o tecido adiposo marrom é composto por adipócitos marrons, que são formados por pequenas gotículas de gordura, uma grande quantidade de mitocôndrias e um núcleo. O número elevado de mitocôndrias faz com que o gasto energético desse tecido seja alto, pois a energia dispendida por essas células é liberada na forma de calor (151,152). Esse tecido, originalmente, tem função protetora; ao longo da vida, sua quantidade no organismo diminui significativamente. Os adipócitos beges são similares morfologicamente e funcionalmente aos adipócitos marrons originais; porém, com origens embrionárias diferentes - por isso a denominação distinta. (153). Foi observado, tanto em um modelo experimental como em pacientes caquéticos um aumento do tecido adiposo marrom (147). Mediadores como o PTHrP, induzem a expressão do coativador PRDM16, que interage com o fator de transcrição PGC1α, estimulando o aumento das proteínas desacopladoras UCP-1 nas mitocôndrias, levando à transformação dos adipócitos brancos em adipócitos beges (153). O aumento desse tecido se deve ao processo de modificação do tecido adiposo branco para o tecido

adiposo marrom, denominado de "*browning*" (28–30). Os principais mecanismos de lipólise associados ao câncer estão ilustrados na Figura 14.

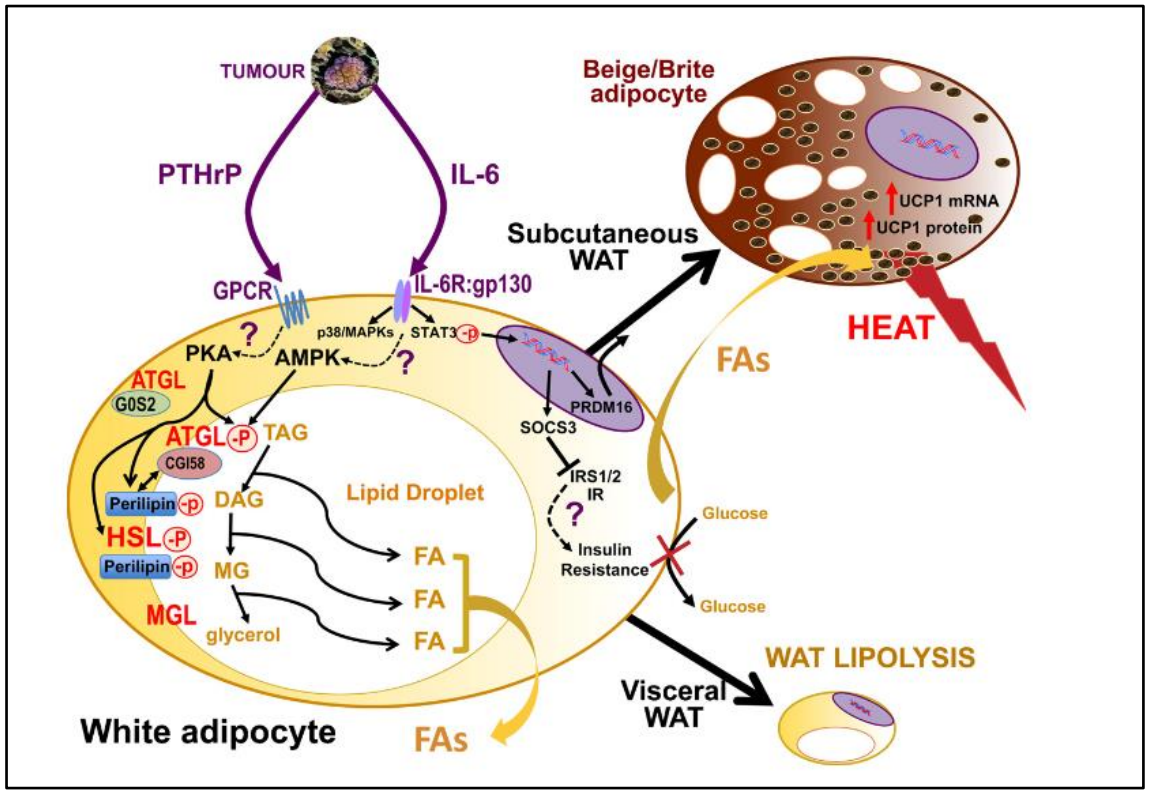

Figure 16 Alterações lipídicas induzidas pela caquexia. Retirado de Tsoli et al (156). Licença para uso: 4610991193302.

1.3.4 Tratamento

Atualmente, o único fármaco aprovado pela Food and Drug Administration (FDA) para o tratamento da caquexia é o acetato de megestrol (derivado sintético da progesterona), lançado em 1993; até o momento, seu mecanismo de ação na caquexia permanece desconhecido (154). Seus principais efeitos são aumento do apetite, um leve aumento de peso corporal, redução de citocinas pró-inflamatórias e aumento de NPY no hipotálamo (155).

O uso da grelina na caquexia parece bastante promissor. A anamorelina, um análogo da grelina, tem sido testada em estudos clínicos de fase III, demonstrado efeitos positivos sobre a caquexia (156–158). Porém, em pacientes com câncer de pulmão de células não pequenas, altos níveis de grelina são associados apenas a pacientes que apresentaram anorexia e não caquexia, demonstrando que o tratamento com grelina e seus análogos pode ser benéfico apenas para uma parte da população oncológica (159). Do mesmo modo, a grelina tem sido testada em diferentes modelos pré-clínicos. Por exemplo, em um modelo experimental de caquexia, o tratamento com grelina e com agonistas do receptor de grelina inibiu a caquexia induzida, tanto pelo câncer, como pelo quimioterápico, cisplatina (160,161).

A caquexia induzida pelo câncer promove resistência à insulina, um dos principais mecanismos de atrofia muscular (131). A metformina, um medicamento utilizado no tratamento do diabetes mellitus tipo 2 (DM2), aumentou o consumo alimentar e prolongou a sobrevida de ratos caquéticos (162). Outro medicamento utilizado anteriormente para DM2, a rosiglitazona, preveniu perda de peso corporal e inibiu a atrofia muscular em um modelo experimental de câncer de cólon (131). Os sensibilizadores de insulina parecem promissores para o tratamento da caquexia; porém, mais estudos são necessários.

Em relação aos tratamentos não farmacológicos, há evidências demonstrando efeitos benéficos do exercício físico na caquexia e também de algumas estratégias nutricionais (163,164). Contudo, o quadro caquético difere da fome e, não somente uma dieta hipercalórica seria a solução (90,165). O uso da suplementação com óleo de peixe rico em ômega-3 é uma das estratégias mais comuns na nutrição oncológica. Todavia, apenas o EPA parece ter algum efeito anticaquético, quando administrado em combinação com outro suplemento ou medicação (166–168). Porém, o uso do ômega-3 como

tratamento coadjuvante na caquexia induzida pelo câncer ainda necessita de mais estudos (169).

De maneira notável, Baracos *et al*. (91) demonstraram (Figura 15) que, embora existam diferentes drogas sendo testadas para o manejo da caquexia associada ao câncer, ainda assim, o manejo nutricional ainda é o mais utilizado para o tratamento da caquexia associada ao câncer.

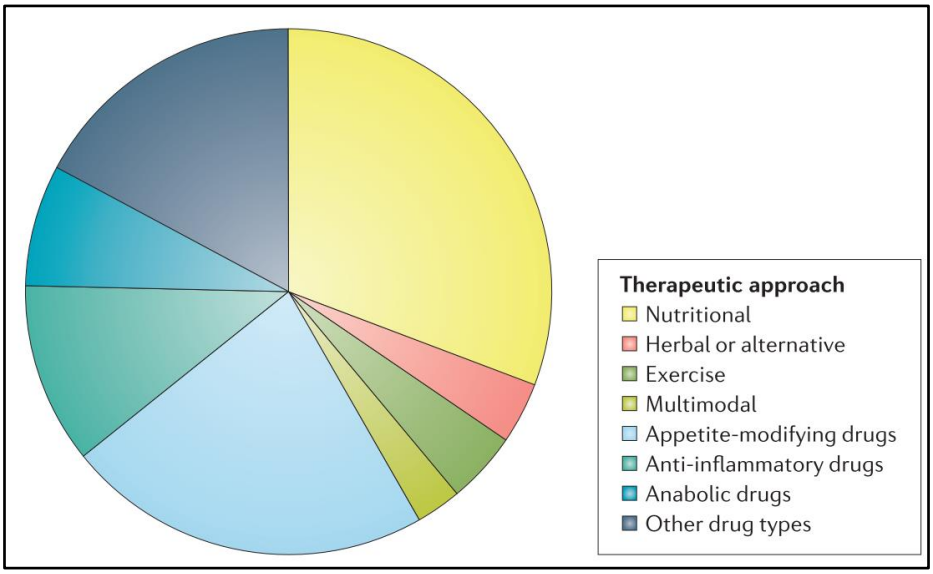

Figura 17 Diferentes abordagens terapêuticas utilizadas na caquexia associada ao câncer. Retirada de Baracos et al (93). Licença para uso: 4610991012214

Uma vez que a caracterização dos receptores FFA é recente, ainda há poucos dados científicos dos seus possíveis mecanismos e alvos terapêuticos. Como descrito anteriormente, ambos receptores têm um efeito marcante sobre a resistência à insulina; porém, em um quadro de obesidade e DM2. Sabendo que a resistência à insulina é um dos principais mecanismos de atrofia muscular e que a inflamação crônica também é um mecanismo do desenvolvimento da caquexia, seria possível sugerir que esses receptores representam alvos terapêuticos para a caquexia induzida pelo câncer.

3 Objetivos

3.1 Objetivo geral

Revisar os efeitos dos ácidos graxos ômega-3 sobre as complicações relacionadas ao câncer e avaliar o papel dos receptores de ácidos graxos livres, FFA1 e FFA4, em um modelo experimental de caquexia associada ao câncer.

3.2 Objetivos específicos

- Realizar uma revisão crítica dos efeitos dos ácidos graxos ômega-3 sobre as complicações relacionadas ao câncer.
- Avaliar os efeitos dos ligantes naturais - ALA e DHA - dos receptores FFA1 e FFA4, dos agonistas sintéticos duais, GW9508 e TUG891, e dos antagonistas seletivos do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre o peso corporal, esplenomegalia, anemia e linfócitos circulantes de camundongos caquéticos induzidos pela linhagem LLC.
- Analisar os efeitos da ativação dos receptores FFA1/FFA4, pelo agonista dual GW9508, sobre os níveis de citocinas, no modelo de caquexia induzida por LLC em camundongos.
- Avaliar os efeitos dos ligantes naturais - ALA e DHA - dos receptores FFA1 e FFA4, dos agonistas sintéticos duais, GW9508 e TUG891, e dos antagonistas seletivos do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre a musculatura esquelética e sobre o tecido adiposo visceral e subcutâneo de camundongos caquéticos induzidos pela linhagem LLC.
- Avaliar os efeitos dos ligantes naturais - ALA e DHA - dos receptores FFA1 e FFA4, dos agonistas sintéticos duais, GW9508 e TUG891, e dos antagonistas

seletivos do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre alterações histológicas da musculatura esquelética e do tecido adiposo do modelo de caquexia induzida por LLC em camundongos.

- Avaliar a expressão de UCP-1 no tecido adiposo no modelo de caquexia induzida por LLC em camundongos, após o tratamento com o agonista dual do FFA1/FFA4, GW9508.
- Avaliar os efeitos dos ligantes naturais - ALA e DHA - dos receptores FFA1 e FFA4, dos agonistas sintéticos duais, GW9508 e TUG891, e dos antagonistas seletivos do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre a atividade locomotora no modelo experimental de caquexia induzida por LLC em camundongos.
- Avaliar os efeitos dos ligantes naturais - ALA e DHA - dos receptores FFA1 e FFA4, dos agonistas sintéticos duais, GW9508 e TUG891, e dos antagonistas seletivos do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre a força e coordenação motora no modelo experimental de caquexia induzida por LLC em camundongos.
- Caracterizar e analisar possíveis alterações cerebrais no modelo de caquexia induzida por LLC em camundongos, utilizando imagens geradas por microPET/CT, após o tratamento com os ligantes naturais – ALA e DHA – e com o agonista sintético dual dos receptores FFA1/FFA4, GW9508.
- Analisar a expressão de FFA1 e FFA4 no tecido adiposo subcutâneo e visceral dos camundongos após a indução de caquexia pelas células LLC.
- Analisar a expressão de FFA1 na musculatura esquelética e no pâncreas de camundongos submetidos à indução de caquexia pela implantação de células LLC, após o tratamento com o agonista dual para FFA1 e FFA4, GW9508.

Capítulo 2

Artigo de revisão

Artigo de revisão publicado na revista *Nutrients*, fator de impacto 4.196 (JCR: 2017), na edição especial "*Effects of Omega-3 Polyunsaturated Fatty Acids and Human Health*". Revista indexada-Qualis A2.

Review

Protective Effects of Omega-3 Fatty Acids in Cancer-Related Complications

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Abstract: Omega-3 polyunsaturated fatty acids (PUFAs) are considered immunonutrients and are commonly used in the nutritional therapy of cancer patients due to their ample biological effects. Omega-3 PUFAs play essential roles in cell signaling and in the cell structure and fluidity of membranes. They participate in the resolution of inflammation and have anti-inflammatory and antinociceptive effects. Additionally, they can act as agonists of G protein-coupled receptors, namely, GPR40/FFA1 and GPR120/FFA4. Cancer patients undergo complications, such as anorexia-cachexia syndrome, pain, depression, and paraneoplastic syndromes. Interestingly, the 2017 European Society for Clinical Nutrition and Metabolism (ESPEN) guidelines for cancer patients only discuss the use of omega-3 PUFAs for cancer-cachexia treatment, leaving aside other cancer-related complications that could potentially be managed by omega-3 PUFA supplementation. This critical review aimed to discuss the effects and the possible underlying mechanisms of omega-3 PUFA supplementation in cancer-related complications. Data compilation in this critical review indicates that further investigation is still required to assess the factual benefits of omega-3 PUFA supplementation in cancer-associated illnesses. Nevertheless, preclinical evidence reveals that omega-3 PUFAs and their metabolites might modulate pivotal pathways underlying complications secondary to cancer, indicating that this is a promising field of knowledge to be explored.

Keywords: omega-3; cancer; nutrition; anorexia-cachexia syndrome; pain; depression; paraneoplastic syndromes

1. Introduction

Bang and Dyerberg investigated the Greenland Eskimo diet in the 1970s in order to determine the reason for why this population had a low prevalence of cardiovascular diseases. The Eskimo diet was composed of seal and whale blubber, containing high protein and low carbohydrate levels, as well as the same amount of fat, when compared to the regular Danish diet [1,2]. The leading cause of the low prevalence of cardiovascular diseases had been attributed to the high dietary contents of omega-3 polyunsaturated fatty acids PUFAs) [3].

Omega-3 PUFAs are classified as essential because they cannot be synthesized by the organism; hence, the consumption of food rich in omega-3, such as fish from cold waters, nuts, and seed oils, is mandatory [4]. The beneficial effects of omega-3 PUFA consumption are likely related to its anti-inflammatory and pro-resolution effects, mainly due to the inhibition of nuclear factor kappa B (NF-κB) and the production of pro-resolution mediators, such as resolvins, protectins, and maresins [5,6]. More recently, two G protein-coupled receptors, called Free Fatty Acid Receptor 1 (FFA1) and Free Fatty Acid Receptor 4 (FFA4), were identified as molecular targets for omega-3 PUFAs [7,8]. When activated, these receptors can promote a number of effects, such as improving the insulin sensibility, inducing adipose tissue browning, promoting analgesia by the release of β-endorphin, controlling energy homeostasis, and diminishing food intake [9–12].

According to GLOBOCAN 2018, a project of the International Agency for Research on Cancer, 18.1 million new cases of cancer and 9.6 million cancer-related deaths worldwide were estimated for the year 2018. For 2020, 17 million new cases are estimated; 66% will live for nearly five years, and at least 40% will live for more than 10 years after diagnosis. Every year, 8.5 million people die from cancer [13]. Lung cancer is the most diagnosed type of cancer for both sexes and the leading cause of death. In males, lung cancer is also the most common and the first cause of death, followed by prostate and colorectal cancers. Among women, breast cancer is the leading type of cancer and the main cause of death, followed by colorectal and lung cancers [13].

As stated by the International Association for the Study of Pain, pain is an “unpleasant sensory and emotional experience, associated with actual or potential tissue damage or described in terms of such damage”, and neuropathic pain is the principal type of pain in cancer patients [14–16]. Cancer pain is the

most common cancer-related complication, reported by approximately 90% of patients. Unfortunately, up to 50% of these patients are poorly treated for this condition. Pain in cancer patients occurs because of the tumor growth itself, metastasis development, or treatment-related adverse effects, such as chemotherapy-induced neurotoxicity. Pain in cancer survivors is also important because any change in this condition can indicate a recurrence of the tumor [14,15,17].

Another important cancer-associated complication is anorexia-cachexia syndrome, which affects up to 85% of cancer patients [18]. This condition is defined as a multifactorial syndrome with muscle atrophy, fat loss, and the progressive defeat of function, leading to a low quality of life, which cannot be reversed by conventional nutritional therapy [19]. Skeletal muscle atrophy and an increase of energy balance occur due to systemic inflammation and a reduced appetite. Particularly, systemic inflammation is the main mechanism for the development of proteolysis, lipolysis, insulin resistance, and a high resting energy expenditure in this condition [20]. Likewise, tumor-derived factors have similar roles as inflammatory cytokines regarding the catabolic effects. Additionally, cancer patients who develop cachexia lose their independence regarding daily chores, leading to a low quality of life [21,22].

The diagnosis of cancer can provoke stress and sadness, leading to major depressive disorder (MDD). However, MDD is not only explained by the emotional impact of the diagnosis. Pro-inflammatory cytokines related to cancer and/or treatment play a key role in cancer-related depression [23]. The prevalence of depression in cancer patients is around four times higher than in the general population, although it does not increase with the severity of the disease. On the other hand, depression is associated with a poor prognosis in cancer patients, mainly due to the low adherence to treatment, which is caused by a lack of family ties and social support, by a history of childhood trauma, and by adverse life experiences [24,25].

Paraneoplastic syndromes involve a wide variety of symptoms related to tumor presence but are not associated with development and malignancy, being a result of the tumor-induced release of hormones or peptides. Unlike the conditions cited above, in general, paraneoplastic syndromes only affect 8% of all cancer patients [26]. Interestingly, the presence of a paraneoplastic syndrome can lead to a cancer diagnosis. Paraneoplastic disorders can have a high mortality rate, but they are manageable and curable after cancer treatment. Due to the rarity of these cases, clinical evidence and guidelines to aid treatment are still lacking when compared to other cancer-related complications [26–28].

Considering that approximately 20% to 80% of cancer patients use dietary supplements after diagnosis [29], the severity of cancer-associated complications, and the beneficial effects of omega-3 PUFAs, the

purpose of this critical review article is to discuss the possible mechanisms and effects of omega-3 PUFA supplementation in principal cancer-related disorders.

2. Omega-3 PUFAs and the Possible Mechanisms of Action in Cancer Complications

Omega-3 PUFAs are essential fatty acids, containing between 18 and 22 carbons, with the first double bond on the third carbon, counting from the omega end. Omega-3 PUFAs comprise three different active molecules: (i) α-linolenic acid (ALA; 18:3n-3), (ii) eicosapentaenoic acid (EPA; 20:5n-3), and (iii) docosahexaenoic acid (DHA; 22:6n-3). ALA is synthesized in plants and can be found in seeds, nuts, and plant oils. EPA and DHA are not synthesized by the organism and can only be found in the flesh of cold-water fish [30]. Interestingly, ALA can be converted to EPA and DHA by several reactions of elongation and desaturation, but these conversions produce small amounts of EPA and DHA in the organism [31].

The omega-6 arachidonic acid (AA; 20:4n-6) and linoleic acid (LA; 18:2n-6) are also essential fatty acids. Notably, both became major components of the cell membrane due to the increase of Western diets, rich in cereals and vegetable oils, containing excessive omega-6 PUFAs and leading to an undesired omega-6/omega-3 ratio of 20:1 [32]. The metabolic pathways of AA and LA share the same enzymes that convert ALA to EPA and DHA, indicating that there is competition between the pathways. In inflammatory processes, membrane phospholipids are cleaved by phospholipase A2 (PLA2) to release AA to the cytoplasm and initiate the production of highly inflammatory eicosanoids (such as prostaglandin E2 and leukotriene B4) by the action of cyclooxygenases and lipoxygenases. The membrane lipid composition modification from an omega-6 PUFA to omega-3 PUFA profile is very important because it increases the production of omega-3-derived mediators, such as thromboxane A3 and prostacyclin I3, which are weaker inducers of inflammation [33]. Supporting this mechanism, a systematic review and meta-analysis demonstrated that omega-3 PUFAs were able to reduce thromboxane B2 blood levels in subjects with a high risk of cardiovascular diseases, along with a decrease of leukotriene B4 in the neutrophils of unhealthy patients [34]. Regarding lymphocyte membranes, an in vitro and pilot clinical study evaluated the fatty acid composition of CD4+T cell membranes after EPA and DHA supplementation. The in vitro analysis showed that EPA or DHA incubation increased the membrane contents of omega-3 PUFAs. Additionally, the pilot clinical study from the same article evaluated the membrane composition of lymphocytes in elderly individuals after six weeks of omega-3 PUFA supplementation and observed a similar omega-3 PUFA-rich

membrane [35]. Additionally, a review article demonstrated that EPA and DHA supplementation are often employed in the nutritional therapy of cancer patients and promotes beneficial effects during cancer treatment due to a membrane modulation [36]. On the other hand, an analysis of the fatty acid composition of the red blood cells of cancer patients showed that there was no difference between the omega-3 PUFAs contents in the membrane of cancer patients and healthy subjects, irrespective of their diet. Interestingly, the same cancer patients showed higher omega-6 PUFA contents and an increased desaturation activity, demonstrating a higher inflammatory profile [37].

The notion that an omega-3 PUFA-enriched membrane could be favorable for disease management was corroborated by the discovery of pro-resolution mediators of inflammation, derived from omega-3 PUFAs. Over the past decade, the identification of resolvins, protectins/neuroprotectins, and maresins was a milestone—currently, it is well-recognized that solving, rather than inhibiting, inflammation is quite an interesting approach for the treatment of a series of chronic illnesses such as cancer.

In acute inflammation, the production of prostaglandins by the action of cyclooxygenases-1 and -2 is essential for blood flow regulation and an increase of endothelial permeability. Additionally, the production of leukotrienes is required for leukocyte migration [38]. Notably, it was believed that all products of the inflammatory process, such as eicosanoids, prostanoids, cytokines, and chemokines, are diluted over time and that the inflammation process would be resolved [39]. Nevertheless, studies demonstrated that a group of lipid pro-resolution mediators, derived from arachidonic acid (AA), namely lipoxins, were crucial to stopping the pro-inflammatory signals, indicating that the resolution of inflammation is an active process [40]. Lipoxins can inhibit the entrance of new neutrophils and stimulate macrophages to clear apoptotic neutrophils [41]. Remarkably, omega-3 PUFAs are crucial for the generation of potent pro-resolution mediators, with similar actions to lipoxins, such as resolvins, protectins, neuroprotectins, and maresins. Resolvins are divided into the series E (RvE) and D (RvD), originating from EPA and DHA, respectively. As for protectins, neuroprotectins and maresins originate from DHA, but maresins are produced only by macrophages [42–44]. These mediators of resolution can decrease the leukocyte infiltration and reduce cellular debris, leading to the cessation of the inflammatory process [44]. Notably, they have been widely investigated, showing beneficial effects in a series of preclinical inflammation models. Regarding the effects of these pro-resolution mediators in cancer, RvD1, RvD2, and RvE1 were capable of reducing the debris-stimulated cancer progression by inducing macrophage phagocytosis and diminishing pro-inflammatory cytokines [45]. Likewise, DHA-derived pro-resolution mediators, such as neuroprotectin D1,

maresin 1, and RvD1 and RvD5, displayed important analgesic effects in a mouse model of postoperative pain after bone fracture when administered after surgery. Nevertheless, the same study demonstrated that DHA administration before surgery partially reduced postoperative pain due to the conversion of DHA to pro-resolution mediators [46]. Concerning the effects of resolution mediators on depression, RvE1 and RvE2 intracerebroventricular (i.c.v.) administration significantly decreased lipopolysaccharide (LPS)-associated depressive behavior via the activation of the resolvin receptor ChemR23 according to the assessment of LPS-induced depression in a mouse model [47]. Similarly, a study of our group revealed the beneficial effects of RvD2 treatment in the depression-like behavior in a mouse model of fibromyalgia [48]. A critical review speculated that the resolution of inflammation is flawed in cancer-cachexia, suggesting that the induction of the resolution process would be beneficial for cancer-cachectic patients [49]. Surprisingly, there are no experimental or clinical studies investigating the effects of pro-resolution mediators in cancer cachexia.

Omega-3 PUFAs can activate G protein-coupled receptors, generating intracellular effects. Firstly, Briscoe et al. (2003) identified the FFA1 receptor, formerly known as the G-protein coupled receptor 40 (GPR40), as a free fatty acid receptor. It was observed that long-chain fatty acids could cause a concentration-dependent increase in intracellular calcium in human embryonic kidney (HEK293) cells expressing FFA1 [7]. The expression of the FFA1 receptor indicates that this receptor is an important molecular target for metabolism control, as observed in the gastrointestinal tract, pancreatic β-cells, and brain [50–52]. Regarding the effects of FFA1 in the metabolism, the activation of this receptor is associated with glucagon-like peptide-1 (GLP-1) and cholecystokinin release [53,54]. More recently, it was observed that the FFA1 receptor is expressed in the melanocortin system, specifically in the neuropeptide Y/Agouti-related peptide (NPY/AgRP) and proopiomelanocortin/cocaine- and amphetamine-regulated transcript (POMC/CART) neurons [55]. Interestingly, the FFA1 expression is upregulated in other tissues under pathological situations, such as periodontitis, which is associated with metabolic syndrome [56]. Regarding the antidiabetic effect, FFA1 has been widely investigated as a molecular target for diabetes, mainly due to the glucose-stimulated insulin secretion via protein kinase C/inositol triphosphate (PKC/IP_3) activation and, consequently, intracellular calcium increase, inducing insulin release [57,58]. In virtue of this effect, TAK-975, a synthetic selective FFA1 agonist, was tested until phase II of clinical trials for diabetes management. Unfortunately, the clinical investigation had been interrupted because patients developed hepatoxicity and liver failure [59,60]. More recently, the role of the FFA1 receptor in the central nervous system has attracted

interest. The activation of this receptor by DHA demonstrated analgesic effects in different experimental pain models [11,12,61,62]. As for FFA1 ligands, long-chain fatty acids are considered endogenous agonists, mainly DHA, but the studies demonstrated that oleic acid is also a potent FFA1 agonist [10,61- 63].

After the identification of FFA1 as a free fatty acid receptor, FFA4, formerly known as G-protein-coupled receptor 120 (GPR120), was also identified as part of this new family of G-protein coupled receptors. On the subject of FFA4 ligands, EPA, ALA, and DHA are considered endogenous ligands, but the latter presents a lesser potency [64]. Similar to FFA1, FFA4 is also activated by long-chain fatty acids and also has metabolic functions [8,65]. Notably, the expression of this receptor can be induced by a fish oil-enriched diet and by the aerobic exercise of different organs [66,67]. Osteoclasts and osteoblasts also express FFA4, and in the presence of high levels of omega-3 fatty acids, it can promote bone formation and inhibit bone resorption [68]. The FFA4 receptor can be found in the taste buds, liver, adipose tissue, intestines, macrophages, and pancreas [69]. Interestingly, the human FFA4 receptor exists in two isoforms: long and short. The long has a 16-residue segment in the third intracellular loop that decouples the receptor from the G protein. However, both isoforms can activate β-arrestin-2, recruiting the transforming growth factor β-activated kinase 1(TAK1)-binding protein 2 (TAB2), which inhibits TAK1, leading to anti-inflammatory effects [65]. As for the main mechanism of action of FFA4, the activation of this receptor leads to $G_{q/11}$ protein activation, stimulating IP_3 and, consequently, increasing intracellular calcium concentration, resulting in hormone secretion. Regarding the effects of FFA4 in obesity, it was observed that FFA4 is localized in NPY-positive neurons, indicating that FFA4 activation by omega-3 PUFAs can decrease appetite, food reward, and anxiety-like behavior [70,71]. At last, the activation of FFA4 induces the browning of the adipose tissue (white adipocytes are transformed into beige adipocytes), indicating another mechanism against the development of obesity [9,72]. Thus, it is tempting to suggest that FFA4 activation by omega-3 PUFAs might interfere with cancer bone metastasis and cachexia, although further studies on this hypothesis are still required.

3. Omega-3 PUFAs as Part of Pharmaconutrition in Cancer Patients

The areas of immunonutrition and pharmaconutrition have emerged due to the impact of nutrients in the organism being greater than the nutrition itself. Nevertheless, pharmaconutrition, which is characterized by nutrient supplementation at pharmacological doses, seems to be more promising than immunonutrition, defined as a nutrient-enriched diet [73]. Malnourished cancer patients can display a diminished response to

cancer therapy, increase in infections, an extension of the length of hospital stay, an augmentation of the risk of postoperative complications, and death [74,75]. Patients may experience mechanical and functional alterations, especially when the tumor is located in the gastrointestinal tract. Additionally, they can display adverse effects related to cancer treatment, such as nausea, vomiting, mucositis, xerostomia, and/or dysphagia [76]. Also, a high inflammatory state in cancer patients might be related to cancer complications, such as depression, cachexia, pain, and paraneoplastic syndromes [77,78]. Immunonutrition with omega-3 PUFAs, glutamine, arginine, and ribonucleotides is often prescribed to cancer patients and is believed to maintain immunocompetence during the treatment [79,80]. Conversely, other clinical randomized trials observed that immune-enhancing diets, when offered to cancer patients, failed to improve the immune response and were no different from standard diets [81–83]. Alternatively, pharmaconutrition is employed as a nutrient supplementation during cancer treatment in order to diminish treatment-related complications. Currently, omega-3 PUFAs can be considered as pharmaconutrients, acting as receptor agonists, modulating molecular pathways, reducing the inflammatory response, increasing the chemotherapy efficacy, and consequently improving the overall survival of cancer patients [84–86]. Curiously, low contents of omega-3 PUFAs in the mammary region seem to contribute to breast cancer multifocality, indicating that omega-3 PUFA supplementation is important for cancer management and prevention [87]. Therefore, omega-3 PUFA-based pharmaconutrition is likely useful for handling cancer-related outcomes.

4. Cancer-Related Pain

Most cancer patients experience different types of pain associated with the disease. Cancer patients often report intense pain, leading to a lower performance status [88]. Pain might be related to tumor localization, but it can also arise due to chemotherapy treatment and/or surgery [89]. Notably, cancer pain comprises inflammatory and neuropathic mechanisms in virtue of tumor mass development [90]. Signaling molecules that are released by the environment are responsible for tissue remodeling and for tumor invasion and metastasis. These molecules can be pro-inflammatory cytokines, chemokines, and growth factors, which are released by cells in order to modulate tumor growth [91]. Additionally, chemo- and radiotherapy induce toxicity and inflammation, evoking painful symptoms, decreasing patients' quality of life and, consequently, diminishing the treatment adherence [92].

Peculiarly, we were not able to find any clinical or experimental studies on omega-3 supplementation for alleviating tumor-related pain. Similarly, clinical evidence of the effects of omega-3 supplementation

in therapy-related pain is still scarce. Preclinical and clinical evidence on the neuroprotective effects of omega-3 PUFAs on chemotherapy-associated pain is provided in Table 1. Supporting the favorable analgesic actions of omega-3 PUFAs, a systematic review demonstrated that a nutritional supplement enriched with fish oil decreased the symptoms of fatigue and pain in patients during chemo- and/or radiotherapy, probably due to weight maintenance and reduced inflammatory status [93,94].

One might dispute the mechanisms underlying the analgesic effects of omega-3 PUFAs in cancer patients. A study, conducted by our research group, demonstrated that an omega-3 PUFA-enriched diet evoked analgesic effects in a mouse model of cyclophosphamide-induced visceral pain due to the overexpression of the FFA1 receptor in the spinal cord [11]. According to studies on other pain models, the activation of the FFA1 receptor induces the release of β-endorphin, noradrenaline, and serotonin, accounting for the analgesic actions of DHA [12,62]. Recently, it was observed that RvD2 decreased cancer pain in an experimental model of oral squamous cell carcinoma, probably via the downregulation of RvD2 receptors in this cancer cell, indicating that the resolution pathways could be suppressed. Another possible mechanism is the inhibition of several members of the transient receptor (TRP) family, such as TRPV1, TRPA1, TRPV3, and TRPV4 by RvD2 [95]. In virtue of the conversion of omega-3 PUFAs in specialized pro-resolution mediations, such as resolvins, protectins, and maresins, it is tempting to suppose that omega-3 PUFA supplementation prior to or during cancer treatment could inhibit or delay the appearance of treatment complications, such as pain and neuropathy.

Table 1. A summary of the articles discussed above regarding the effects of omega-3 PUFAs in cancer and cancer-treatment complications.

Authors	Cancer-related Complication	Species	Cancer type	Treatment Scheme	Major Outcome
Hershmann et al., 2015 [96]	Aromatase-inhibitor associated arthralgia	Human	Breast cancer	3.3 g [1] FO (560 mg EPA + DHA; 40:20)	Decreased pain, evaluated by the [2] BPI between the baseline and week 24 ($p <$ 0.01)

Shen et al., 2018 [97]	Aromatase-inhibitor associated arthralgia	Human	Breast cancer (obese)	3.3 g FO (560 mg EPA + DHA; 40:20)	Pain reduction in [3] BMI > 30 kg/m² patients (p = 0.02)
Martínez et al., 2018 [98]	Aromatase-inhibitor musculoskeletal symptoms (AIMSS)	Human	Breast cancer	460 mg EPA+DHA 12.5 mg hydroxytyrosol 50 g curcumin	Decrease of the BPI total score after 30 days (p = 0.011)
Ghroreishi et al., 2012 [99]	Paclitaxel-induced neuropathy	Human	Breast cancer	640 mg FO (54% DHA + 10% EPA)	70% did not develop neuropathy no pain score assessed
Maschio et al., 2018 [100]	Bortezomib-related neuropathy	Human	Multiple myeloma	Neuronorm® (400 mg DHA + 600 mg ALA)	Pain failed to increase significantly (p = 0.33)
Freitas et al., 2016 [11]	Cyclophosphamide-induced hemorrhagic cystitis	Mice	-	20% FO-enriched diet or 1 µmol/kg i.p.	Decrease in spontaneous pain behavior and abdominal allodynia (p < 0.01)
Ye et al., 2018 [95]	Oral and paw cancer pain	Mice	Oral squamous cell carcinoma	RvD1 (100 ng or 200 ng) or RvD2 (100 ng or 200 ng) i.p.	RvD2 inhibited thermal and mechanical pain;

[1] FO: Fish oil; [2] BPI: Brief pain inventory; [3] BMI: body mass index.

5. Anorexia-Cachexia Syndrome

The use of omega-3 PUFA supplementation to treat anorexia-cachexia syndrome is commonly employed in cancer patients. However, the beneficial effects of these molecules for this complication are still questionable [101]. Particularly, there are no treatment plans for anorexia-cachexia syndrome in virtue of the multifactorial characteristics of this syndrome [102], demonstrating that an open discussion on the benefits of low-cost management, such as fish oil supplementation, is extremely important for clinical practice.

Regarding clinical evidence, studies using fish oil supplements, as a source of omega-3 fatty acids, demonstrated distinct effects on the development of cancer cachexia. For instance, head and neck cancer patients receiving an omega-3-enriched nutritional supplement received no benefits concerning cachexia features [103]. However, fish oil supplementation stabilized the weight of gastrointestinal cancer patients [104,105]. Interestingly, a recent systematic review evaluated the effects of fish oil for cachexia in advanced cancer, concluding that clinical evidence is still uncertain due to a weak methodology and a large variation of fish oil dosages. However, fish oil supplementation could benefit postoperative recovery and reduce complications, such as impaired wound healing and infections [106].

Remarkably, the plasmatic contents of omega-6/omega-3 and ALA/EPA were associated with muscle atrophy in cachectic cancer patients, indicating that these molecules may participate in the development of cancer cachexia [107,108]. A recent review article demonstrated that, during the last 23 years, in 31 clinical trials, cancer patients had some benefit from the use of omega-3 supplementation, mainly EPA. Regardless of the quantity of clinical and preclinical studies, this review concluded that the mechanisms underlying the benefits of omega-3 supplementation for cancer cachexia are still unknown [109]. Notably, EPA supplementation improved body weight and lean body mass in cancer patients by modulating circulating inflammatory markers, such as tumor necrosis factor (TNF), interleukin-1 β (IL-1β), interleukin-6 (IL-6), and interferon- γ (IFN-γ), demonstrating an inhibitory effect on inflammatory parameters related to muscle atrophy and lipolysis [110]. Additionally, Pappalardo et al. (2015) reviewed the issue of EPA as an anti-inflammatory agent, concluding that EPA supplementation has a positive effect in stabilizing lean body

mass when compared to standard supplementation by diminishing the levels of C-reactive protein, IL-6, and TNF [111]. Two systematic reviews drew different conclusions regarding omega-3 supplementation. Colomer et al. (2007) demonstrated grade-B evidence (reasonable scientific evidence suggesting that the clinical benefits overcome the potential risks), suggesting that a dose of at least 1.5 g per day of EPA/DHA is related to enhanced clinical, biological, and quality of life parameters [112]. On the other hand, Mazzotta and Jeney (2008) showed that EPA and DHA failed to show significant clinical benefits related to body weight, lean body mass, survival, and life quality [113]. Concerning DHA alone, there is no clinical evidence demonstrating the effects of this molecule in cancer-associated cachexia.

Preclinical evidence provides further knowledge about the favorable effects of omega-3 supplementation in cancer-associated cachexia. In an in vivo model of cancer cachexia, EPA supplementation decreased the expression of zinc-α2-glycoprotein (ZAG), a lipolytic factor, in white and brown adipose tissue [114], demonstrating another mechanism of action related to EPA supplementation in cachexia. Regarding the EPA anti-lipolytic effect, Du et al. (2015) observed similar effects in the S180 ascitic cancer model after treatment with EPA derived from the starfish, *Asterias amurensis*, which was due to a reduction of ZAG, adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), peroxisome proliferator-activated receptor gamma coactivator 1-α (PGC-1α), and mitochondrial uncoupling protein 2 (UCP-2) expressions [115]. Notably, EPA supplementation alone reversed some aspects of the Lewis lung cancer-cachexia mouse model. However, training exercise, combined with EPA, promoted a stronger recovery after the development of lung cancer-associated cachexia in mice, mainly via the inhibition of the ubiquitin-proteasome system [116]. Concerning the effects of DHA alone in preclinical models of cancer-associated cachexia, the existing evidence is still scarce. In a mouse model of chemotherapy-induced body weight loss, a DHA-enriched diet was able to prevent body weight loss and to reduce glycerol release, indicating that DHA also had an anti-lipolytic effect [117]. In reference to the use of ALA in cancer cachexia, one study evaluated the effect of this molecule in a rat model of cancer cachexia using Oro Inca Oil (derived from *Sacha inchi* oil, a plant from the Andes), which is rich in ALA. Tumor-bearing rats receiving Oro Inca oil displayed an improvement of body weight, diminished IL-6, and TNF circulating levels, as well as decreased triacylglyceride (TAG) levels, demonstrating that ALA-rich oil also has anti-cachectic effects [118]. For a better comprehension of the scenery regarding cachexia management, Table 2 summarizes the data discussed above regarding the use of omega-3 PUFAs in this syndrome.

In light of the literature data, the use of omega-3 supplementation likely represents an interesting treatment option for cancer cachexia management in virtue of its anti-inflammatory, anti-lipolytic, and anti-catabolic actions. Since there are no studies involving cancer-associated cachexia and pro-resolution mediators, it is reasonable to propose that omega-3 conversion to pro-resolution mediators underlie part of the beneficial effects of omega 3 PUFAs in cancer. However, the absence of studies analyzing the role of free fatty acid receptors in cancer-induced cachexia limits our knowledge of the beneficial mechanisms mediating the omega-3 effects on this cancer-associated complication, indicating that novel studies are still required.

Table 2. A brief summary of the selected articles on the use of omega-3 PUFAs as a treatment for cancer-related cachexia.

Authors	Cancer-related complication	Species	Cancer Type	Treatment Scheme	Major Outcome
Hanai et al., 2018 [103]	Cachexia-anorexia syndrome	Human	Head and neck squamous cell carcinoma	Prosure ® (1056 mg EPA)	No significant difference among experimental groups
Persson et al., 2005 [104]	Cachexia-anorexia syndrome	Human	Advanced gastrointestinal cancer	30 mL/d [1] FO (4.9g EPA + 3.2 g DHA)	FO stabilized weight in 27% patients
Shirai et al., 2017 [105]	Cachexia-anorexia syndrome	Human	Advanced gastrointestinal cancer	Prosure ® (1.1 g EPA + 0.5 g DHA)	Increase of body weight and lean body mass (p = 0.002/p < 0.001)
Werner et al., 2017 [119]	Cachexia-anorexia syndrome	Human	Pancreatic cancer	6.9g EPA/13.6 g	No significant differences

				DHA in 100g or 8.5g EPA/ 12.3 g DHA in 100g	between omega-3 PUFA treatments
Solis-Martínez et al., 2018 [110]	Cachexia-anorexia syndrome	Human	Head and neck squamous cell carcinoma	2 g EPA	Weight and ^{2} LBM maintenance
Hajjaji et al., 2012 [117]	Chemotherapy-induced cachexia	Rat	Chemically-induced tumor + doxorubicin treatment	DHA-enriched diet (80g/kg diet)	DHA diet avoided weight loss
Schissel et al., 2015 [118]	Cancer-associated cachexia	Rat	Breast carcinoma (Walker 256 cell line)	53.6% EPA + DHA or 54.4% ALA	ALA and EPA improved weight gain (cachectic vs. cachectic + omega-3 $p <$ 0.05)
Du et al., 2015 [115]	Cancer-related cachexia	Mice	Sarcoma (S180 cell line)	42% EPA + 6.8% DHA	Decreased lipolysis and increased body weight ($p <$ 0.001)
Penna et al., 2011 [116]	Cancer-related cachexia	Mice	Lewis lung carcinoma	EPA (0.5 g/kg) or EPA (0.5 g/kg) + exercise	EPA + exercise significantly improved muscle weight ($p < 0.05$)

Muzio et al., 2016 [120]	Cachexia in vitro model	Human	Lung adenocarcinoma	50 µM EPA + DHA	Myoblast formation

[1] FO: Fish oil; [2] LBM: lean body mass.

6. Major Depression Disorder (MDD)

Depression commonly occurs in cancer patients, affecting between 5% and 60% of oncological patients [121]. Pro-inflammatory cytokines, such as TNF, IL-1β, and IFN-γ, are released by the tumor–host interaction and can reach the hypothalamus, inducing a depression-like behavior. These cytokines can also stimulate the expression of serotonin and noradrenaline uptake transporters, leading to a diminished quantity of these neurotransmitters in the central nervous system [122]. Additionally, cancer-related depression can be evoked by cancer-independent mechanisms due to the impact of cancer diagnosis and stress [123]. Another probable mechanism, responsible for the development of cancer-related depression, is the upregulation of the leptin receptor, as observed in the gastric tissue of depressive gastric cancer patients, demonstrating that leptin may be involved in the pathogenesis of cancer-associated depression [124].

Regarding the participation of omega-3 in cancer-associated depression, it was observed that, in newly diagnosed Japanese lung cancer patients, the ALA and total omega-3 consumption was inversely associated with the development of depression. Nonetheless, EPA and DHA intake displayed no interaction whatsoever [125]. On the contrary, in the same population, a higher serum DHA was correlated with minor depression, but the authors indicated that the study had several limitations that could influence this conclusion [126]. Surprisingly, no further clinical trial investigated whether omega-3 PUFA supplementation could, to some extent, benefit cancer-related depression.

An overall analysis of the literature regarding the effects of omega-3 PUFAs in depression revealed controversial data. For instance, a meta-analysis study suggested that omega-3 supplementation is beneficial for depressed individuals [127]. On the other hand, Appleton et al. (2016) claimed that there is insufficient evidence to determine that omega-3 supplementation could be useful for depression treatment [128]. Nonetheless, Smith et al. (2017) demonstrated that a low-dose of DHA reduced depression and, interestingly, decreased insomnia, leading to a much-elevated quality of life [129]. In a cross-sectional analysis, a moderate dietary intake of omega-3 PUFAs was associated with a lower prevalence of depression [130]. It was noteworthy that patients with schizophrenia and depression displayed a low

erythrocyte omega-3 index [131]. Similarly, Bigornia et al. (2016) demonstrated that the erythrocyte omega-3 index was inversely associated with depression in patients with elevated oxidative stress biomarkers [127]. Additionally, Müller et al. (2015) stated that a lack of omega-3 PUFAs in the brain could lead to a higher probability of developing depression and anxiety disorders [128]. Therefore, these studies support the hypothesis that omega-3 PUFAs can at least play a partial role in brain diseases, such as MDD.

As for the depression-like behavior related to cancer treatment, Orchard et al. (2016) considered that omega-3 supplementation could be beneficial for chemotherapy-induced cognitive alterations, such as depression [132]. Regarding preclinical evidence, rats submitted to repeated LPS administration displayed depressive-like behaviors, associated with decreased levels of monoamines besides an increase of apoptotic markers in the hippocampus and prefrontal cortex. It was noteworthy that fish oil supplementation reversed all of these effects, displaying an important anti-inflammatory action [133]. Fat-1 is a transgenic mouse model that endogenously transforms omega-6 to omega-3 by expressing *C. elegans fat-1* gene-encoding omega-3 desaturase [134]. Strikingly, these animals displayed benefits regarding neuroinflammation and oxidative stress in the depression model induced by LPS. Interestingly, this study showed that LPS depression induces a pro-inflammatory M1 phenotype in hyperactive microglia, and endogenous omega-3 shifted this phenotype to an anti-inflammatory phenotype M2 [135].

According to Larrieu and Layé (2018), omega-3 PUFAs exhibit neuroprotective effects in the development of brain diseases, such as depression and anxiety, particularly by the sensing activity of free fatty acid receptors [136]. The effects, modulated by an activation of the free fatty acid receptors, mainly FFA1, were only observed in experimental models, but it is possible to surmise that the activation of this receptor might be important for depression management. For instance, the repeated administration of GW9508, an FFA1 receptor agonist, was able to diminish the immobility time of mice during a forced swimming test. Additionally, the same study showed that DHA and AA levels were decreased in the hippocampus after the behavioral test, although the FFA1 expression remained unaltered [137]. Corroborating these data, Aizawa et al. (2016) demonstrated that FFA1 knockout mice exhibited augmented anhedonia and altered levels of serotonin, dopamine, and noradrenaline in the hippocampus [138]. Similar effects were observed in FFA1 knockout female mice. In addition to the increased anhedonia, female mice presented reduced maternal care, such as negligence and infanticide; reduced locomotor activity; and decreased social interaction [139]. Concerning the effects of pro-resolution mediators in depression,

molecules derived from DHA were further investigated rather than EPA-derived molecules, revealing the antidepressant effects of resolvins of the D series when given i.c.v. in rodent models [47,140–142].

Taking into account the effects promoted by omega-3 PUFAs in different animal models of depression and depressed individuals, as summarized in Table 3, it is possible to assume that omega-3 and omega-3-derived mediators can play an important role in MDD, possibly via the activation of FFA1 or resolution pathways. Considering the evidence mentioned above, the management of depression in cancer patients could very well be another indication supporting omega-3 PUFA supplementation in the treatment of cancer. An improvement of the depression symptoms by omega 3 or related fatty acid dosing might contribute to a better response to cancer treatment and an overall life quality improvement of the affected individuals.

Table 3. A brief summary of the selected articles using omega-3 PUFAs as treatment for depression.

Authors	Clinical or experimental condition	Species	Treatment scheme	Major outcome
Chhetry et al, 2016 [143]	MDD	Human	4 g [1] FO (1.6 g EPA + 0.8 g DHA)	Improved MDD-related white matter deficiency
Smith et al., 2017 [129]	MDD	Human	260 mg or 520 mg DHA	54% of patients showed a reduction of depression severity ≥ 50%
Wu et al., 2018 [144]	Chemotherapy-induced depression	Rat	1.5 g/kg omega-3 PUFAs (34% EPA + 24 % DHA)	PUFAs inhibited depressive-like behaviors ($p < 0.001$)
Dang et al., 2018 [133]	LPS-induced depression	Rat	1.5 g/kg omega-3 PUFAs (34%	Omega-3 PUFAs decreased depressive behavior ($p <$ 0.001)

			EPA + 24 % DHA)	
Nishinaka et al., 2014[137]	Behavioral despair paradigm	Mice	GW9508 (1.0, 10 or 25 µg/mouse) i.c.v	FFA1 activation decreased immobility in a tail suspension test ($p < 0.05$)
Deyama et al., 2017 [140]	LPS-induced depression	Mice	RvD1 (10 ng i.c.v.) or RvD2 (10 ng i.c.v.)	Both treatments inhibited depressive-like behaviors ($p < 0.005$)
Deyama et al., 2018 [141]	LPS-induced depression	Mice	RvE3 (10 and 100 ng i.c.v.)	Inhibition of depressive behavior ($p < 0.005$)
Ishikawa et al., 2017 [142]	Chronic unpredictable stress-related depression	Mice	RvD1 (10 ng i.c.v.) or RvD2 (10 ng i.c.v.)	Both treatments inhibited depressive behavior for 24 h ($p < 0.05$)

[1]FO: Fish oil.

7. Paraneoplastic Syndromes

Paraneoplastic syndromes are multiple clinical complications that are related to tumor metabolites, but they are considered rare. These syndromes are categorized as neurological, endocrinological, hematological, dermatological, and rheumatological complications [26]. Interestingly, it is possible to suppose that complications, such as anorexia-cachexia syndrome and cancer pain, could also be classified as paraneoplastic syndromes due to their pathophysiology mechanism.

Endocrine paraneoplastic syndromes occur due to the interaction of substances released by the tumor cells originating from endocrine or neuroendocrine cells, which are distributed through different parts of the human body. Non-endocrine tumor cells can also liberate substances, promoting similar symptoms and sharing the same clinical features [27]. The most common endocrine paraneoplastic syndromes are hypercalcemia, the syndrome of inappropriate antidiuretic hormone secretion (SIADH), and Cushing's syndrome. On the other hand, other complications, such as non-islet cell tumor hypoglycemia, gynecomastia, acromegaly, hypertension, ovarian hyperstimulation syndrome, hyperprolactinemia, hyperthyroidism, and secretory diarrhea, are considered rare endocrine paraneoplastic syndromes [145]. As

for hypercalcemia, this syndrome develops due to the protein related to the parathyroid hormone-related peptide (PTHrP) released by the tumor, stimulating bone resorption and leading to higher levels of serum parathyroid hormone (PTH) and osteoclast hyperactivity. Interestingly, the same PTHrP secretion induces the browning of the adipose tissue, leading to an increase of energy expenditure and leading to the cachectic state [146]. One might presume that omega-3 supplementation could be beneficial for hypercalcemia-associated bone resorption because it was observed that, in an animal model of apical periodontitis, omega-3 supplementation reduced bone resorption by the downregulation of the inflammatory cells influx [147]. Moreover, the combination of omega-3 supplementation and exercise in postmenopausal healthy women promoted diminished serum PTH levels, leading to an improvement of skeletal health [148]. Nevertheless, there is no evidence linking paraneoplastic tumor-induced hypercalcemia and omega-3, as well as in relation to other kinds of paraneoplastic endocrine syndromes.

Neurological paraneoplastic syndromes are a consequence of the production of tumor antibodies, known as *onconeural antibodies*, which react with the nervous system, promoting damage [149]. Interestingly, neurological paraneoplastic syndromes are detected before cancer diagnosis and can help patient prognosis by a premature treatment initiation. The most common neurological paraneoplastic syndromes are encephalopathies, neuropathies, encephalomyelitis, cerebellar degeneration, myelitis, myasthenic syndrome, myasthenia gravis, neuromyotonia, dermatomyositis, and stiff person syndrome [149,150]. Limbic encephalitis is one of the most common paraneoplastic syndromes and develops by a reaction to Anti-Hu (HuD antigen; small cell lung carcinoma), Anti-Ma2 (Ma proteins; germ-cell tumors), or Anti-NMDA (N-methyl-D-aspartate; teratomas), which are mainly characterized by neuroinflammation. In relation to general encephalitis, omega-3 supplementation demonstrated neuroprotective effects against traumatic brain injury by reducing microglial activation and regulating the Toll-like receptor 4 (TLR4)/NF-kB signaling pathway [151]. Again, there is no evidence linking the use of omega-3 supplementation and neurological paraneoplastic syndromes, but it is possible to presume that omega-3 could be beneficial in some kinds of neuropathy. This notion is based on the available literature related to omega-3 supplementation and other types of neuropathy. In a mouse model of diabetic neuropathy, fish oil supplementation, and daily systemic administration of RvE1 and RvD1 reversed thermal hypoalgesia, mechanical allodynia, reduced motor, and sensory nerve conduction and decreased the innervation of cornea and skin [152]. Similar effects were observed by Yorek et al. (2016) in a mouse model of diabetic neuropathy, when fish oil and RvD1 promoted benefits related to the development of neuropathy but not to

diabetes itself [153]. Interestingly, this last study demonstrated that the combination with fish oil and salsalate promoted similar anti-inflammatory effects when compared to RvD1 or fish oil alone, probably due to the acetylation of cyclooxygenases, resulting in an increase of RvD1 and leading to the resolution of the inflammatory process.

Rheumatologic paraneoplastic syndromes develop in a similar way to endocrine paraneoplastic syndromes [154]. This kind of paraneoplastic syndrome is also considered rare, and it can appear two years before cancer diagnosis. The most common rheumatological paraneoplastic syndromes are hypertrophic osteoarthropathy, polyarthritis, tumor-induced osteomalacia, and cancer-associated myositis [155]. As for the other paraneoplastic syndromes, there is no evidence concerning the effects of omega-3 supplementation in these alterations. Therefore, it is only possible to assume that omega-3 could be beneficial based on the existing literature of similar alterations. In dogs suffering from osteoarthritis, fish oil supplementation attenuated oxidative stress, and inflammatory markers after the dietary intervention, but no pain assessment was evaluated [156]. Additionally, DHA supplementation in rheumatoid arthritis patients significantly reduced the clinical and biochemical symptoms of inflammation [157]. Regarding cancer-associated myositis, an in vitro study evaluated the effects of DHA in LPS-induced inflammation in myoblast cells (C2C12 myotubes), and it was observed that 30 mM of DHA prevented lipotoxicity and skeletal muscle inflammation [158]. Tumor-induced osteomalacia is caused by tumors that secrete fibroblast growth factor 23 (FGF23), inducing hypophosphatemia, leading to a reduced osteoblast differentiation and matrix mineralization [159]. Literature related to tumor-induced osteomalacia and omega-3 fatty acids is nonexistent. Nevertheless, in renal transplant patients, EPA/DHA intake decreased FGF23 circulating levels [160]. Thus, omega-3 supplementation might control tumor-associated hypophosphatemia, consequently reducing the development of osteomalacia.

Regarding dermatological paraneoplastic syndromes, they can represent 1% of the first diagnostic in cancer patients. Skin alterations related to neoplasms are caused by vascular alterations or a high differentiation of keratinocytes/fibroblasts. The more rapidly the skin manifestation appears, the higher the probability that it will be associated with cancer [161]. Commonly, paraneoplastic dermatological syndromes are acanthosis nigricans, dermatomyositis, erythroderma, leukocytoclastic vasculitis, paraneoplastic pemphigus, polymyalgia rheumatica, and Sweet's syndrome. Concerning the skin paraneoplastic alterations, there is no evidence regarding the effects of omega-3 supplementation, even though the importance of omega-3 dietary supplementation for skin health is well-known [162]. Moreover,

concerning wound-healing effects, omega-3 supplementation promotes beneficial therapeutic effects in healthy subjects [163].

Finally, hematological paraneoplastic syndromes are rarely symptomatic and usually related to the presence of a tumor. The most common hematological paraneoplastic syndromes are eosinophilia, granulocytosis, pure red cell aplasia, and thrombocytosis. Generally, hematological alterations are often induced by an increase of pro-inflammatory cytokine circulation. In dogs suffering from osteoarthritis, fish oil reduced circulating basophils and monocytes, but it failed to prevent lymphocyte, neutrophil, and eosinophil alterations [156]. Considering the role of omega-3 pro-resolution mediators, resolvins and protectins promoted the reduction of circulating eosinophils [164].

There is limited preclinical or clinical evidence on the effects of omega-3 supplementation in cancer patients that develop paraneoplastic syndromes. However, it may be possible to presume that omega-3 supplementation could be beneficial—or, in any case, harmless—for cancer patients with paraneoplastic syndromes and that a higher daily intake of omega-3 fatty acids could prevent the evolution of future paraneoplastic alterations. Finally, the lack of evidence regarding omega-3 supplementation effects on paraneoplastic syndromes indicates the need for future cancer-associated complications research.

8. Literature Trends Regarding Omega-3 PUFAs and Cancer Complications

With the aim of visualizing the existing evidence discussed in this review, we used the VOSViewer software (Leiden University, Netherlands) to build a co-occurrence network of terms, based on publications retrieved from PubMed, using the following keywords: “cancer pain”, “cancer depression”, “cancer cachexia”, and “paraneoplastic syndrome”, each combined with “omega-3”. From the database, containing data of about 297 articles (original and review articles), VOSViewer extracted the most relevant and repeated terms in the retrieved publications (considering title, keywords, and abstract). To be considered, a term had to appear in at least 10 articles.

As can be seen in Figure 1, the circles represent terms as they appear in the database, and their size is related to the number of articles in which they appear. A line connecting two terms indicates that both terms occur in the same article, and the thicker the lines, the larger the number of articles sharing both terms, which can be considered a measure of the association strength between the terms. The distribution of the

circles in the network is also related to the co-occurrence so that the circles are positioned close to others, depending on the association strength between the terms they represent.

The network also represents clusters of terms, depicted in different colors. Such clusters allow for an understanding of the structure of the publications set because they result from a process that considers the similarity of the co-occurrences of the terms. It is possible to observe that the main clusters are green and red. The green ones comprise terms related to omega-3 PUFAs and their use in diseases, such as cancer and mental disorders. Curiously, the term “systematic review” appears in the same cluster, demonstrating that there are more reviews on these subjects. The red cluster comprises terms related to “nutritional therapy” in cancer and other complications, such as “chronic pain”. Importantly, the word “inhibitor” appears in red but is more distant from the red cluster core, probably due to its co-occurrence with terms in other clusters, although it is related to “supplementation” and “nutritional support”. The third major cluster is in blue, and it is directly related to pharmacological and molecular terms, such as “tumour growth” and “tumour”. Additionally, ALA appears in the same cluster, demonstrating that the use of ALA can be related to the "inhibition" of molecular pathways. Near these terms, one can observe that “tumour growth” is in yellow. Despite being located near to the blue cluster, due to its close relation to other terms that are also linked to the British form of writing “tumour”, it belongs to the yellow cluster, which is positioned in a more central area. The location of the yellow cluster results from the co-occurrence of its terms with many others belonging to other clusters. The fourth cluster is in purple, and its terms are linked to protein and muscle metabolism. Moreover, this type of analysis demonstrates the problem of standardizing some terms throughout the scientific literature, as it can be seen in “pufa” and “pufas”, emerging as two different terms. The organization of the existing literature data in such a co-occurrence network clearly shows the distance between cancer complications, such as “chronic pain” (red cluster), “depressive symptom” (green cluster), and “protein degradation” (purple cluster), which demonstrate that there is a lack of publications addressing the relationship between omega-3 PUFAs and the cancer-related complications revised in this review article.

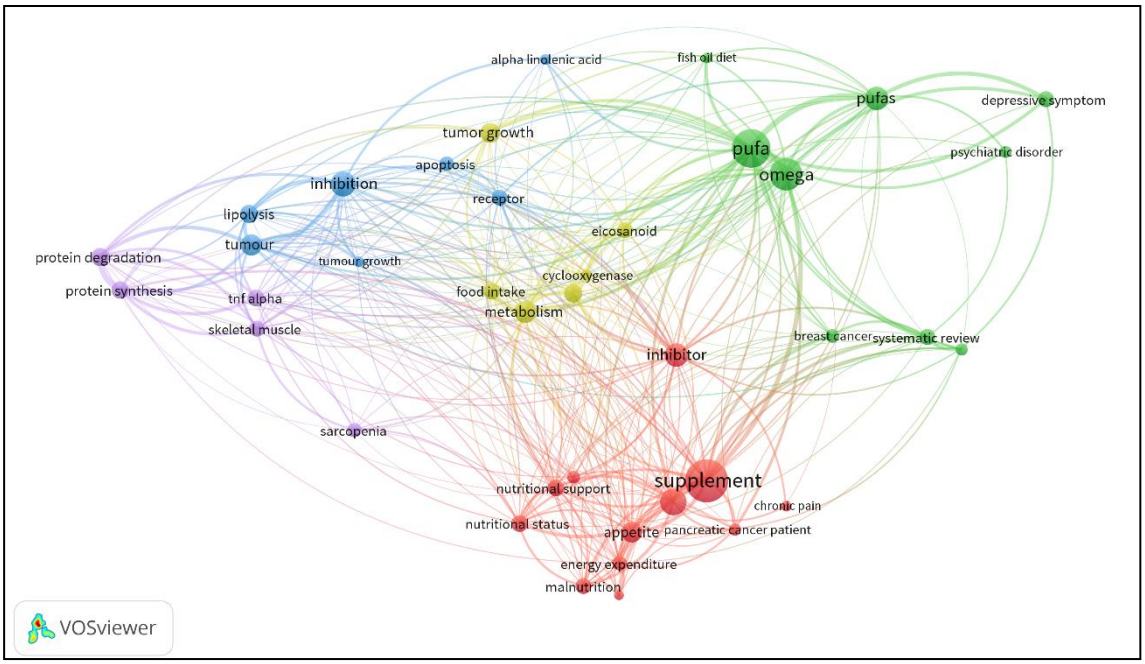

Figure 1. A co-occurrence network of terms based on publication data retrieved from PubMed using keywords that reproduce the search performed for this review article. The circle sizes represent the number of articles featuring the corresponding term; the links between the circles represent the co-occurrence of terms in the same articles; and the line thickness is dependent on the number of articles sharing the terms. Different colors identify clusters composed by closely related terms.

9. Conclusions

Since the 1970s, omega-3 PUFAs have been a subject of multiple investigations due to their ability to suppress inflammatory processes. In recent years, it has become possible to identify some of the mechanisms of action of these molecules besides the potential molecular targets. As can be seen throughout this critical review, an omega-3 supplementation is widely employed in cancer patients, mainly as an adjunctive treatment. The identification of pro-resolution mediators derived from omega-3 fatty acids opened up a variety of therapeutic possibilities for different pathologies. More recently, the identification of free fatty acid receptors as therapeutic targets for omega-3 PUFAs also revealed a plethora of beneficial opportunities. Importantly, it is imperative to emphasize that, in 2018, Omegaven® (Fresenius Kabi, Germany) was approved by the Federal Drug Administration (FDA) for parenteral nutrition in cholestasis, demonstrating that it is important to consider omega-3 fatty acids as a therapeutic option that is related to all regulatory functions, similar to a new pharmaceutical drug [165].

Regarding the beneficial effects of omega-3 fatty acids in cancer-related complications, additional studies are still needed, mainly randomized clinical trials with omega-3 supplementation, due to the deficiency of clinical literature evidence. There is also a lack of scientific evidence regarding whether omega-3 PUFAs are able to significantly prevent or address cancer-related complications, depending on the cancer stage, from dysplasia to carcinoma and metastasis. In Figure 2, we attempted to summarize the possible pathways by which omega-3 PUFAs might promote favorable effects in cancer-related complications, such as pain, paraneoplastic syndrome, depression, and cachexia-anorexia syndrome. The definition of these mechanisms might also account for the development of novel strategies based on omega-3 PUFAs, contributing to an improvement of the life quality of cancer patients in the near future.

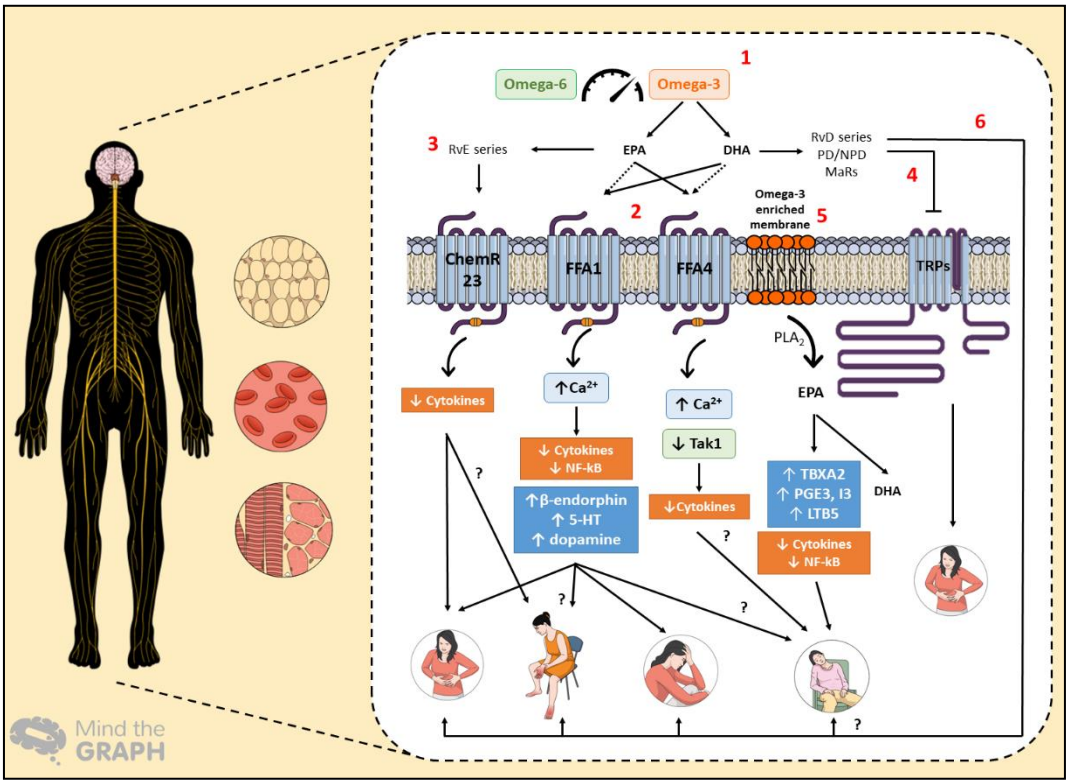

Figure 2. The proposed mechanisms of action for omega-3 polyunsaturated fatty acid (PUFA) intake in cancer-related complications, which affect the central and peripheral nervous system, besides adipose tissue and skeletal muscle. Hematological changes depict the switched production of systemic inflammatory mediators under cancer progression. (1) The balance between omega-3 and omega-6 PUFAs is essential for the generation of pro-resolution mediators, the sensing of free fatty acid receptors, and membrane modulation. (2) Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can stimulate Free Fatty Acid

Receptor 1 (FFA1) and Free Fatty Acid Receptor 4 (FFA4) receptors in different ways, leading to anti-inflammatory effects. FFA1 activation can evoke analgesia and antidepressant effects. Additionally, this receptor could have benefits in relation to paraneoplastic syndromes, such as neuropathy and cachexia-anorexia syndrome, represented herein by fatigue. (3) E-series resolvins (RvE) promotes anti-inflammatory effects via ChemR23 activation, likely contributing to the alleviation of painful symptoms. They might also induce beneficial effects in other paraneoplastic syndromes. (4) The inhibitory effects of D-series resolvins (RvD) on transient receptor (TRP) channels could also produce favorable effects on cancer-related pain. (5) As for the omega-3 PUFA membrane enrichment, the production of thromboxane A2 (TBXA2), prostaglandins E3 and I3 (PGE3/I3), and leukotriene B5 (LTB5) promotes anti-inflammatory effects that prompt cachectic patients' welfare. (6) Finally, RvD-series, protectins/neuroprotectins (PD/NPD), and maresins (MaRs) have beneficial effects on pain and depression; thus, they could similarly relieve the signs and symptoms of paraneoplastic and cachexia-anorexia syndromes.

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References

1. Dyerberg, J.; Bang, H.O.; Hjorne, N. Fatty Acid composition of the plasma lipids in Greenland Eskimos. *Am. J. Clin. Nutr.* **1975**, *28*, 958–966.
2. Bang, H.O.; Dyerberg, M.D.; Sinclair, H.M. The composition of the Eskimo western food in north. *Am. J. Clin. Nutr.* **1980**, *33*, 2657–2661.
3. Watanabe, Y.; Tatsuno, I. Omega-3 polyunsaturated fatty acids for cardiovascular diseases: Present, past and future. *Expert Rev. Clin. Pharm.* **2017**, *10*, 865–873, doi:10.1080/17512433.2017.1333902.
4. Simopoulos, A.P. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. *Nutrients* **2016**, *8*, 128.
5. Fang, X.; Ge, K.; Song, C.; Ge, Y.; Zhang, J. Biochemical and Biophysical Research Communications Effects of n-3PUFAs on autophagy and inflammation of hypothalamus and body weight in mice. *Biochem. Biophys. Res. Commun.* **2018**, *501*, 927–932, doi:10.1016/j.bbrc.2018.05.084.
6. Spencer, L.; Mann, C.; Metcalfe, M.; Webb, M.B.; Pollard, C.; Spencer, D. The effect of omega-3 FAs on tumour angiogenesis and their therapeutic potential. *Eur. J. Cancer* **2009**, *45*, 2077–2086, doi:10.1016/j.ejca.2009.04.026.
7. Briscoe, C.P.; Tadayyon, M.; Andrews, J.L.; Benson, W.G.; Chambers, J.K.; Eilert, M.M. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. *J. Biol. Chem.* **2003**, *278*, 11303–11311.
8. Hirasawa, A.; Tsumaya, K.; Awaji, T.; Katsuma, S.; Adachi, T.; Yamada, M. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. *Nat. Med.* **2005**, *11*, 90–94.
9. Quesada-López, T.; Cereijo, R.; Turatsinze, J.V.; Planavila, A.; Cairó, M.; Gavaldà-Navarro, A. The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. *Nat. Commun.* **2016**, *7*, 13479.
10. Oliveira, V.; Marinho, R.; Vitorino, D.; Santos, G.A.; Moraes, J.C.; Dragano, N. Diets containing alpha-linolenic (n-3) or oleic (n-9) fatty acids rescues obese mice from insulin resistance. *Endocrinology* **2015**, *156*, 4033–4046.
11. Freitas, R.D.S.; Costa, K.M.; Nicoletti, N.F.; Kist, L.W.; Bogo, M.R.; Campos, M.M. Omega-3 fatty acids are able to modulate the painful symptoms associated to cyclophosphamide-induced-hemorrhagic cystitis in mice. *J. Nutr. Biochem.* **2016**, *27*, 219–232..

12. Nakamoto, K.; Nishinaka, T.; Ambo, A.; Mankura, M.; Kasuya, F.; Tokuyama, S. Possible involvement of β-endorphin in docosahexaenoic acid-induced antinociception. *Eur. J. Pharmacol.* **2011**, *666*, 100–104..
13. Bray, F.; Ferlay, J.; Soerjomataram, I.; Siegel, R.L.; Torre, L.A.; Jemal, A. Global Cancer Statistics 2018: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancer in 185 Countries. *Cancer J. Clin.* **2018**, *68*, 394–424.
14. Neufeld, N.J.; Elnahal, S.M.; Alvarez, R.H. Cancer pain: A review of epidemiology, clinical quality and value impact. *Future Oncol.* **2017**, *13*, 833–841.
15. Goyal, A.; Bhatnagar, S. Neuropathic pain in cancer. *Ann. Palliat. Med.* **2014**, *3*, 1–3.
16. Bennet, M.I.; Kaasa, S.; Barke, A.; Korwisi, B.; Riek, W.; Treede, R.-D. The IASP classification of chronic pain for ICD-11: Chronic secondary headache and orofacial pain. *Pain* **2019**, *160*, 38–44.
17. Candido, K.D.; Kusper, T.M.; Knezevic, N.N. New Cancer Pain Treatment Options. *Curr. Pain Headache Rep.* **2017**, *21*, 1–12.
18. Argilés, J.M.; Stemmler, B.; López-Soriano, F.J.; Busquets, S. Inter-tissue communication in cancer cachexia. *Nat. Rev. Endocrinol.* **2018**, doi:10.1038/s41574-018-0123-0.
19. Fearon, K.; Strasser, F.; Anker, S.D.; Bosaeus, I.; Bruera, E.; Fainsinger, R.L. Definition and classification of cancer cachexia: An international consensus. *Lancet Oncol.* **2011**, *12*, 489–495..
20. Argilés, J.M.; Busquets, S.; Stemmler, B.; López-Soriano, F.J. Cachexia and sarcopenia: Mechanisms and potential targets for intervention. *Curr. Opin. Pharm.* **2015**, *22*, 100–106.
21. Seelaender, M.; Laviano, A.; Busquets, S.; Püschel, G.P.; Margaria, T.; Batista, M.L., Jr. Inflammation in Cachexia. *Mediators Inflamm.* **2015**, *2015*, 2–4.
22. Argilés, J.M.; Busquets, S.; Stemmler, B.; López-Soriano, F.J. Cancer cachexia: Understanding the molecular basis. *Nat. Rev. Cancer* **2014**, *14*, 754–762, doi:10.1038/nrc3829.
23. Bortolato, B.; Hyphantis, T.N.; Valpione, S.; Perini, G.; Maes, M.; Morris, G. Depression in cancer: The many biobehavioral pathways driving tumor progression. *Cancer Treat Rev.* **2017**, *52*, 58–70, doi:10.1016/j.ctrv.2016.11.004.
24. Sotelo, J.L.; Musselman, D.; Nemeroff, C. The biology of depression in cancer and the relationship between depression and cancer progression. *Int. Rev. Psychiatry* **2014**, *26*, 16–30.
25. Watts, S.; Prescott, P.; Mason, J.; McLeod, N.; Lewith, G. Depression and anxiety in ovarian cancer: A systematic review and meta-analysis of prevalence rates. *BMJ Open* **2015**, *5*, e007618.

26. Pelosof, L.C.; Gerber, D.E. Paraneoplastic Syndromes: An Approach to Diagnosis and Treatment. *Mayo Clin. Proc.* **2010**, *85*, 838–54, doi:10.4065/mcp.2010.0099.
27. Efthymiou, C.; Spyratos, D.; Kontakiotis, T. Endocrine paraneoplastic syndromes in lung cancer. *Hormones* **2018**, *17*, 351–358.
28. Viau, M.; Renaud, M.C.; Grégoire, J.; Sebastianelli, A.; Plante, M. Paraneoplastic syndromes associated with gynecological cancers: A systematic review. *Gynecol. Oncol.* **2017**, *146*, 661–671, doi:10.1016/j.ygyno.2017.06.025.
29. Marian, M.J. Dietary Supplements Commonly Used by Cancer Survivors: Are There Any Benefits? *Nutr. Clin. Pract.* **2017**, *32*, 607–627.
30. Saini, R.K.; Keum, Y. Omega-3 and omega-6 polyunsaturated fatty acids: Dietary sources, metabolism, and significance—A review. *Life Sci.* **2018**, *203*, 255–267.
31. Simopoulos, A.P.; DiNicolantonio, J.J. The importance of a balanced ω-6 to ω-3 ratio in the prevention and management of obesity. *Open Hear* **2016**, *3*, e000385.
32. Simopoulos, A.P. Evolutionary aspects of diet, the omega-6/omega-3 ratio and genetic variation: Nutritional implications for chronic diseases. *Biomed. Pharmacother.* **2006**, *60*, 502–507.
33. Simopoulos, A.P. The importance of the ratio of omega-6/omega-3 essential fatty acids. *Biomed. Pharmacother.* **2002**, *56*, 365–379.
34. Jiang, J.; Li, K.; Wang, F.; Yang, B.; Fu, Y. Effect of Marine-Derived n-3 Polyunsaturated Fatty Acids on Major Eicosanoids: A Systematic Review and Meta-Analysis from 18 Randomized Controlled Trials. *PLoS ONE* **2016**, *11*, e0147351.
35. Fan, Y.; Fuentes, N.R.; Hou, T.Y.; Barhoumi, R.; Li, X.C.; Deutz, N.E.P. Remodelling of primary human CD4+ T cell plasma membrane order by n-3 PUFA. *Br. J. Nutr.* **2018**, *119*, 163–75.
36. Fuentes, N.R.; Kim, E.; Fan, Y.; Chapkin, R.S. Molecular Aspects of Medicine Omega-3 fatty acids, membrane remodeling and cancer prevention. *Mol. Asp. Med.* **2018**, *2017*, 79–91, doi:10.1016/j.mam.2018.04.001.
37. Amézaga, J.; Arranz, S.; Urruticoechea, A.; Ugartemendia, G.; Larraioz, A.; Louka, M. Altered Red Blood Cell Membrane Fatty Acid Profile. *Nutrients* **2018**, *10*, 1853.
38. Gilroy, D.W.; Bailey, D.B. Lipid mediators in immune regulation and resolution. *Br. J. Pharmacol.* **2019**, *2*, 1–15.

39. Serhan, C.N.; Savill, J. Resolution of inflammation: The beginning programs the end. *Nat. Immunol.* **2005**, *6*, 1191–1197.

40. Maddox, B.J.E.; Serhan, C.N. Lipoxin A4 and B4 Are Potent Stimuli for Human Monocyte Migration and Adhesion: Selective Inactivation by Dehydrogenation and Reduction. *J. Exp. Med.* **1996**, *183*, 137–146.

41. Chandrasekharan, J.; Sharma-Walia, N. Lipoxins: Nature's way to resolve inflammation. *J. Inflamm. Res.* **2015**, *8*, 181–92.

42. Serhan, C.N.; Yacoubian, S. Anti-Inflammatory and Proresolving Lipid Mediators. *Annu. Rev. Pathol.* **2008**, *3*, 279–312.

43. Zhang, M.J.; Spite, M. Resolvins: Anti-inflammatory and proresolving mediators derived from omega-3 polyunsaturated fatty acids. *Annu. Rev. Nutr.* **2012**, *32*, 203–227.

44. Serhan, C.N.; Chiang, N.; Dalli, J.; Levy, B.D. Lipid Mediators in the Resolution of Inflammation. *Cold Spring Harb. Perspect. Biol.* **2015**, *7*, a016311.

45. Sulciner, M.L.; Serhan, C.N.; Gilligan, M.M.; Mudge, D.K.; Chang, J.; Gartung, A. Resolvins suppress tumor growth and enhance cancer therapy. *J. Exp. Med.* **2018**, *215*, 115–140.

46. Zhang, L.; Terrando, N.; Xu, Z.; Bang, S.; Jordt, S.; Baker, O.J. Distinct Analgesic Actions of DHA and DHA-Derived Specialized Pro-Resolving Mediators on Post-operative Pain After Bone Fracture in Mice. *Front. Pharmacol.* **2018**, *9*, 412.

47. Deyama, S.; Shimoda, K.; Suzuki, H.; Ishikawa, Y. Resolvin E1/E2 ameliorate lipopolysaccharide-induced depression-like behaviors via ChemR23. *Psychopharmacology* **2018**, *235*, 329–336.

48. Klein, C.P.; Sperotto, N.D.M.; Maciel, I.S.; Leite, C.E.; Souza, A.H.; Campos, M.M. Effects of D-series resolvins on behavioral and neurochemical changes in a fibromyalgia-like model in mice. *Neuropharmacology* **2014**, *86*, 57–66.

49. Seelaender, M.; Batista, M.; Lira, F.; Silverio, R.; Rossi-Fanelli, F. Inflammation in cancer cachexia: To resolve or not to resolve (is that the question?). *Clin. Nutr.* **2012**, *31*, 562–566, doi:10.1016/j.clnu.2012.01.011.

50. Ma, D.; Tao, B.; Warashina, S.; Kotani, S.; Lu, L.; Kaplamadzhiev, D.B. Expression of free fatty acid receptor GPR40 in the central nervous system of adult monkeys. *Neurosci. Res.* **2007**, *58*, 394–401.

51. Edfalk, S.; Steneberg, P.; Edlund, H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. *Diabetes* **2008**, *57*, 2280–2287.

52. Itoh, Y.; Kawamata, Y.; Harada, M.; Kobayashi, M.; Fujii, R.; Fukusumi, S. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. *Nature* **2003**, *422*, 173–176, doi:10.1038/nature01478.

53. Gorski, J.N.; Pachanski, M.J.; Mane, J.; Plummer, C.W.; Souza, S.; Thomas-Fowlkes, B.S. GPR40 reduces food intake and body weight through GLP-1. *Am. J. Physiol. Endocrinol. Metab.* **2017**, *313*, E37–E47, doi:10.1152/ajpendo.00435.2016.

54. Liou, A.; Lu, X.; Sei, Y.; Zhao, X.; Pechhold, S.; Carrero, R. The G-Protein Coupled Receptor GPR40 Directly Mediates Long-Chain Fatty Acid Induced Secretion of Cholecystokinin. *Gastroenterology* **2011**, *140*, 903–912.

55. Dragano, N.R.V.; Solon, C.; Ramalho, A.F.; Moura RFDe Razolli, D.S.; Christiansen, E. Polyunsaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation. *J. Neuroinflamm.* **2017**, *14*, 91.

56. Li, G.; Robles, S.; Lu, Z.; Li, Y.; Krayer, J.W.; Leite, R.S. Upregulation of free fatty acid receptors in periodontal tissues of patients with metabolic syndrome and periodontitis. *J. Periodontal Res.* **2018**, doi:10.1111/jre.12636.

57. Gravena, C.; Mathias, P.; Ashcroft, S.J. Acute effects of fatty acids on insulin secretion from rat and human islets of Langerhans. *J. Endocrinol.* **2002**, *173*, 73–80.

58. Ichimura, A.; Hasegawa, S.; Kasubuchi, M.; Kimura, I. Free fatty acid receptors as therapeutic targets for the treatment of diabetes. *Front. Pharmacol.* **2014**, *5*, 236.

59. Yashiro, H.; Tsujihata, Y.; Takeuchi, K.; Hazama, M.; Johnson, P.R.V.; Rorsman, P. The Effects of TAK-875, a Selective G Protein-Coupled Receptor 40/Free Fatty Acid 1 Agonist, on Insulin and Glucagon Secretion in Isolated Rat and Human Islets. *J. Pharmacol. Exp. Ther.* **2012**, *340*, 483–489.

60. Burant, C.; Viswanathan, P.; Marcinak, J.; Cao, C.; Vakilynejad, M. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: A phase 2, randomised, double-blind, placebo-controlled trial. *Lancet* **2012**, *379*, 1403–1411.

61. Harada, S.; Haruna, Y.; Aizawa, F.; Matsuura, W.; Nakamoto, K.; Yamashita, T. Involvement of GPR40, a long-chain free fatty acid receptor, in the production of central post-stroke pain after global cerebral ischemia. *Eur. J. Pharmacol.* **2014**, *744*, 115–123.

62. Nakamoto, K.; Nishinaka, T.; Sato, N.; Aizawa, F.; Yamashita, T.; Mankura, M. The activation of supraspinal GPR40/FFA1 receptor signalling regulates the descending pain control system. *Br. J. Pharmacol.* **2015**, *172*, 1250–1262.

63. Liotti, A.; Cosimato, V.; Mirra, P.; Calì, G.; Conza, D.; Secondo, A.; Luongo, G.; Terracciano, D.; Formisano, P.; Beguinot, F.; et al. Oleic acid promotes prostate cancer malignant phenotype via the G protein-coupled receptor FFA1/GPR40. *J. Cell. Physiol.* **2018**, *233*, 7367–7378.

64. Alexander, S.P.H.; Christopoulos, A.; Davenport, A.P.; Kelly, E.; Marrion, N.V.; Peters, J.A. The Concise Guide TO Pharmacology 2017/18:G protein-coupled receptors. *Br. J. Pharmacol.* **2017**, *174*, 17–129.

65. Ulven, T.; Christiansen, E. Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. *Annu. Rev. Nutr.* **2015**, *35*, 239–263, doi:10.1146/annurev-nutr-071714-034410.

66. Gaspar, R.; Veiga, C.; Bessi, M.; Dátilo, M.; Sant'Ana, M.; Rodrigues, P. Unsaturated fatty acids from flaxseed oil and exercise modulate GPR120 but not GPR40 in the liver of obese mice: A new anti-inflammatory approach. *J. Nutr. Biochem.* **2019**, *66*, 52–62, doi:10.1016/j.jnutbio.2018.12.003.

67. Dátilo, M.N.; Ramos, M.; Formigari, G.; Rodrigues, P.; de Moura, L.; da Silva, A. Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor. *Sci. Rep.* **2018**, *8*, 14318.

68. Ahn, S.H.; Park, S.; Baek, J.; Lee, S.; Baek, W.; Lee, S. Formation and Suppresses Bone Resorption in the Presence of Elevated n-3 Fatty Acid Levels. *Endocrinology* **2016**, *4*, 2621–2635.

69. Im, D.S. Functions of omega-3 fatty acids and FFA4 (GPR120) in macrophages. *Eur. J. Pharmacol.* **2016**, *785*, 36–43.

70. Cintra, D.; Ropelle, E.; Moraes, J.; Pauli, J.; Morari, J.; de Souza, C. Unsaturated Fatty Acids Revert Diet-Induced Hypothalamic Inflammation in Obesity. *PLoS ONE* **2012**, *7*, e30571.

71. Auguste, S.; Fisette, A.; Fernandes, M.F.; Hryhorczuk, C.; Poitout, V.; Alquier, T. Central Agonism of GPR120 Acutely Inhibits Food Intake and Food Reward and Chronically Suppresses Anxiety-Like Behavior in Mice. *Int. J. Neuropsychopharmacol.* **2016**, *19*, pyw014.

72. Schilperoort, M.; van Dam, A.D.; Hoeke, G.; Shabalina, I.G.; Okolo, A.; Hanyaloglu, A.C. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. *EMBO Mol. Med.* **2018**, *10*, e8047, doi:10.15252/emmm.201708047.

73. Jones, N.E.; Heyland, D.K. Pharmaconutrition: A new emerging paradigm. *Curr. Opin. Gastroenterol.* **2008**, *24*, 215–222.

74. Virizuela, J.A.; Camblor-Álvarez, M.; LML-P; Grande, E.; Álvarez-Hernández, J.; Sendrós-Madroño, M.J. Nutritional support and parenteral nutrition in cancer patients: An expert consensus report. *Clin. Transl. Oncol.* **2018**, *20*, 619–629.

75. Ryan, A.M.; Power, D.G.; Daly, L.; Cushen, S.J.; Ní, E. Cancer-associated malnutrition, cachexia and sarcopenia: The skeleton in the hospital closet 40 years later. *Proc. Nutr. Soc.* **2016**, *75*, 199–211.

76. Gangadharan A, Choi SE, Hassan A, Ayoub NM, Balwani S, Kim YH, et al. Protein calorie malnutrition, nutritional intervention and personalized cancer care. *Oncotarget* **2017**, *8*, 24009–24030.

77. Mcmillan, D.C. The systemic inflammation-based Glasgow Prognostic Score: A decade of experience in patients with cancer. *Cancer Treat. Rev.* **2013**, *39*, 534–540, doi:10.1016/j.ctrv.2012.08.003.

78. Diakos, C.I.; Charles, K.A.; Mcmillan, D.C.; Clarke, S.J. Cancer-related inflammation and treatment effectiveness. *Lancet Oncol.* **2014**, *15*, 493–503.

79. Talvas, J.; Garrait, G.; Goncalves-mendes, N.; Rouanet, J. Immunonutrition stimulates immune functions and antioxidant defense capacities of leukocytes in radiochemotherapy-treated head & neck and esophageal cancer patients: A double-blind randomized clinical trial. *Clin. Nutr.* **2015**, *34*, 810–817, doi:10.1016/j.clnu.2014.12.002.

80. Hamza, N.; Darwish, A.; Reilly, D.A.O.; Denton, J.; Sheen, A.J.; Chang, D. Perioperative Enteral Immunonutrition Modulates Systemic and Mucosal Immunity and the Inflammatory Response in Patients with Periampullary Cancer Scheduled for Pancreaticoduodenectomy A Randomized Clinical Trial. *Pancreas* **2015**, *44*, 41–52.

81. Tanaka, Y.; Takahashi, T.; Yamaguchi, K.; Osada, S. Elemental diet plus glutamine for the prevention of mucositis in esophageal cancer patients receiving chemotherapy: A feasibility study. *Support Care Cancer* **2016**, *24*, 933–941.

82. Mudge, L.A.; Watson, D.I.; Smithers, B.M.; Isenring, E.A.; Smith, L.; Jamieson, G.G. Multicentre factorial randomized clinical trial of perioperative immunonutrition versus standard nutrition for patients undergoing surgical resection of oesophageal cancer. *Br. J. Surg.* **2018**, *105*, 1262–1272.

83. Klek, S.; Scislo, L.; Walewska, E.; Choruz, R.; Galas, A. Enriched enteral nutrition may improve short-term survival in stage IV gastric cancer patients: A randomized, controlled trial. *Nutrition* **2017**, *36*, 46–53, doi:10.1016/j.nut.2016.03.016.

84. Martins, E.; Oliveira, A.C.D.M.; Pizato, N.; Muniz-junqueira, M.I.; Magalhães, K.G.; Nakano, E.Y. The effects of EPA and DHA enriched fish oil on nutritional and immunological markers of treatment naïve breast cancer patients: A randomized double-blind controlled trial. *Nutr. J.* **2017**, *16*, 71.

85. Chagas, T.R.; Borges, D.S.; Oliveira PFDe Mocellin, M.C.; Barbosa, A.M.; Camargo, C.Q. Oral fish oil positively influences nutritional-inflammatory risk in patients with haematological malignancies during chemotherapy with an impact on long-term survival: A randomised clinical trial. *J. Hum. Nutr. Diet.* **2017**, *30*, 681–692.

86. Bougnoux, P.; Hajjaji, N.; Ferrasson, M.N.; Giraudeau, B.; Couet, C.; Floch, O.L. Improving outcome of chemotherapy of metastatic breast cancer by docosahexaenoic acid: A phase II trial. *Br. J. Cancer* **2009**, *101*, 1978–1985, doi:10.1038/sj.bjc.6605441.

87. Ouldamer, L.; Goupille, C.; Vildé, A.; Arbion, F.; Body, G. N-3 Polyunsaturated Fatty Acids of Marine Origin and Multifocality in Human Breast Cancer. *PLoS ONE* **2016**, *11*, e0147148, doi:10.1371/journal.pone.0147148.

88. Caraceni, A.; Portenoy, R. An international survey of cancer pain characteristics and syndromes. *Pain* **1999**, *82*, 263–74.

89. Lara-Solares, A.; Ahumada Olea, M.; Basantes Pinos A de los, Á.; Bistre Cohén, S.; Bonilla Sierra, P.; Duarte Juárez, E.R. Latin-American guidelines for cancer pain management. *Pain Manag.* **2017**, *7*, 287–298, doi:10.2217/pmt-2017-0006.

90. Falk S, Dickenson AH. Pain and Nociception: Mechanisms of Cancer-Induced Bone Pain. *J. Clin. Oncol.* **2014**, *32*, 1647–1654.

91. Elinav, E.; Nowarski, R.; Thaiss, C.A.; Hu, B.; Jin, C.; Flavell, R.A. Inflammation-induced cancer: Crosstalk between tumours, immune cells and microorganisms. *Nat. Rev. Cancer* **2013**, *13*, 759–771.

92. Shinko, D.; Diakos, C.I.; Clarke, S.J.; Charles, K.A. Cancer-Related Systemic Inflammation: The Challenges and Therapeutic Opportunities for Personalized Medicine. *Clin. Pharmacol. Ther.* **2017**, *102*, 599–610.

93. Silva J, d.A.P.; Fabre M, E.d.S.; Waitzberg, D.L. Omega-3 supplements for patients in chemotherapy and/or radiotherapy: A systematic review. *Clin. Nutr.* **2015**, *34*, 359–366, doi:10.1016/j.clnu.2014.11.005.

94. Trabal, J.; Leyes, P.; Forga, M.; Maurel, J. Potential usefulness of an EPA-enriched nutritional supplement on chemotherapy tolerability in cancer patients without overt malnutrition. *Nutr. Hosp.* **2010**, *25*, 736–740.

95. Ye, Y.; Scheff, N.; Bernabé, D.; Salvo, E.; Ono, K.; Cheng, L. Anti-cancer and analgesic effects of resolvin D2 in oral squamous cell carcinoma. *Neuropharmacology* **2018**, *139*, 182–193.

96. Hershman, D.L.; Unger, J.M.; Crew, K.D.; Awad, D.; Dakhil, S.R.; Gralow, J. Randomized Multicenter Placebo-Controlled Trial of Omega-3 Fatty Acids for the Control of Aromatase Inhibitor—Induced Musculoskeletal Pain: SWOG S0927. *J. Clin. Oncol.* **2015**, *33*, 1910–1917.

97. Shen, S.; Unger, J.M.; Crew, K.D.; Till, C.; Greenlee, H.; Gralow, J. Omega-3 fatty acid use for obese breast cancer patients with aromatase inhibitor-related arthralgia (SWOG S0927). *Breast Cancer Res. Treat.* **2018**, *172*, 603–610, doi:10.1007/s10549-018-4946-0.

98. Martínez, N.; Herrera, M.; Frías, L.; Provencio, M.; Pérez-Carrión, R.; Díaz, V. A combination of hydroxytyrosol, omega-3 fatty acids and curcumin improves pain and inflammation among early stage breast cancer patients receiving adjuvant hormonal therapy: Results of a pilot study. *Clin. Transl. Oncol.* **2019**, *21*, 489–498, doi:10.1007/s12094-018-1950-0.

99. Ghoreishi, Z.; Esfahani, A.; Djazayeri, A.; Djalali, M.; Golestan, B.; Ayromlou, H. Omega-3 fatty acids are protective against paclitaxel-induced peripheral neuropathy: A randomized double-blind placebo controlled trial. *BMC Cancer* **2012**, *12*, 355.

100. Maschio, M.; Zarabla, A.; Maialetti, A.; Marchesi, F.; Giannarelli, D.; Gumenyuk, S. Prevention of Bortezomib-Related Peripheral Neuropathy with Docosahexaenoic Acid and α-Lipoic Acid in Patients with Multiple Myeloma: Preliminary Data. *Integr. Cancer Ther.* **2018**, *17*, 1115–1124.

101. Lavriv, D.S.; Neves, P.M.; Ravasco, P. Should omega-3 fatty acids be used for adjuvant treatment of cancer cachexia? *Clin. Nutr. ESPEN* **2018**, *25*, 18–25, doi:10.1016/j.clnesp.2018.02.006.

102. Mattox, T.W. Cancer Cachexia: Cause, Diagnosis, and Treatment Causes of CC. *Nutr. Clin. Pract.* **2017**, *5*, 599–606.

103. Hanai, N.; Terada, H.; Hirakawa, H.; Suzuki, H.; Nishikawa, D.; Beppu, S. Prospective randomized investigation implementing immunonutritional therapy using a nutritional supplement with a high blend ratio of ω-3 fatty acids during the perioperative period for head and neck carcinomas. *Jpn. J. Clin. Oncol.* **2018**, *48*, 356–61.

104. Persson, C.; Glimelius, B.; Rönnelid, J.; Nygren, P. Impact of fish oil and melatonin on cachexia in patients with advanced gastrointestinal cancer: A randomized pilot study. *Nutrition* **2005**, *21*, 170–178.

105. Shirai, Y.; Okugawa, Y.; Hishida, A.; Ogawa, A.; Okamoto, K. Fish oil-enriched nutrition combined with systemic chemotherapy for gastrointestinal cancer patients with cancer cachexia. *Sci. Rep.* **2017**, *7*, 4826.

106. Ries, A.; Trottenber, P.; Elsner, F.; Stiel, S.; Haugen, D.; Kaasa, S.A systematic review on the role of fish oil for the treatment of cachexia in advanced cancer: An EPCRC cachexia guidelines project. *Palliat. Med.* **2011**, *26*, 294–304.

107. Kitagawa, M.; Haji, S.; Amagai, T. Elevated Serum AA/EPA Ratio as a Predictor of Skeletal Muscle Depletion in Cachexic Patients with Advanced Gastro-intestinal Cancers. *In Vivo* **2017**, *31*, 1003–1009.

108. Murphy, R.A.; Mourtzakis, M.; Chu, Q.S.; Reiman, T.; Mazurak, V.C. Skeletal Muscle Depletion Is Associated with Reduced Plasma (n-3) Fatty Acids in Non-Small. *J. Nutr.* **2010**, *140*, 1602–1606.

109. Gorjao, R.; Miguel, C.; Duarte, T.; Serdan, A.; Leonardo, V.; Diniz, S. New insights on the regulation of cancer cachexia by N-3 polyunsaturated fatty acids. *Pharmacol. Ther.* **2019**, *196*, 117–134, doi:10.1016/j.pharmthera.2018.12.001.

110. Solís-martínez, O.; Plasa-carvalho, V.; Phillips-sixtos, G. Effect of Eicosapentaenoic Acid on Body Composition and Inflammation Markers in Patients with Head and Neck Squamous Cell Cancer from a Public Hospital in Mexico. *Nutr. Cancer* **2018**, *70*, 663–670, doi:10.1080/01635581.2018.1460678.

111. Pappalardo, G.; Almeida, A.; Ravasco, P. Eicosapentaenoic acid in cancer improves body composition and modulates metabolism. *Nutrition* **2015**, *31*, 549–555.

112. Colomer, R.; Moreno-Nogueira, J.; García-Luna, P.; García-Peris, P.; García-de-Lorenzo, A.; Zarazaga, A. Systematic Review n-3 Fatty acids, cancer and cachexia: A systematic review of the literature. *Br. J. Nutr.* **2007**, *97*, 823–831.

113. Mazzotta, P.; Jeney, C.M. Anorexia-Cachexia Syndrome: A Systematic Review of the Role of Dietary Polyunsaturated Fatty Acids in the Management of Symptoms, Survival, and Quality of Life. *J. Pain Symptom Manag.* **2008**, *37*, 1069–1077, doi:10.1016/j.jpainsymman.2008.06.005.

114. Russell, S.T.; Tisdale, M.J.Ã. Effect of eicosapentaenoic acid (EPA) on expression of a lipid mobilizing factor in adipose tissue in cancer cachexia. *Prostaglandins Leukot. Essent Fat. Acids* **2005**, *72*, 409–414.

115. Du, L.; Yang, Y.; Wang, Y.; Xue, C. EPA-enriched phospholipids ameliorate cancer-associated cachexia mainly via inhibiting lipolysis. *Food Funct.* **2015**, *6*, 3652–3662.

116. Penna, F.; Busquets, S.; Pin, F.; Toledo, M.; Baccino, F.M.; López-Soriano, F.J. Combined approach to counteract experimental cancer cachexia: Eicosapentaenoic acid and training exercise. *J. Cachexia Sarcopenia Muscle* **2011**, *2*, 95–104.

117. Hajjaji, N.; Couet, C.; Besson, P.; Bougnoux, P. DHA Effect on Chemotherapy-Induced Body Weight Loss: An Exploratory Study in a Rodent Model of Mammary Tumors. *Nutr. Cancer* **2012**, *61*, 37–41.

118. Schiessel, D.L.; Yamazaki, R.K.; Kryczyk, M.; Coelho, I.; Yamaguchi, A.A.; Pequito, D.C.T. α-Linolenic Fatty Acid Supplementation Decreases Tumor Growth and Cachexia Parameters in Walker 256 Tumor-Bearing Rats. *Nutr. Cancer* **2015**, *67*, 839–846.

119. Werner, K.; Küllenberg de Gaudry, D.; Taylor, L.A.; Keck, T.; Unger, C.; Hopt, U.T. Dietary supplementation with n-3-fatty acids in patients with pancreatic cancer and cachexia: Marine phospholipids versus fish oil—A randomized controlled double-blind trial. *Lipids Health Dis.* **2017**, *16*, 104.

120. Muzio, G.; Ricci, M.; Traverso, N.; Monacelli, F.; Oraldi, M.; Maggiora, M. 4-Hydroxyhexenal and 4-hydroxynonenal are mediators of the anti-cachectic effect of n-3 and n-6 polyunsaturated fatty acids on human lung cancer cells. *Free Radic. Biol. Med.* **2016**, *99*, 63–70, doi:10.1016/j.freeradbiomed.2016.07.031.

121. Caruso, R.; Nanni, M.; Riba, M.; Sabato, S.; Mitchell, A.; Croce, E. Depressive spectrum disorders in cancer: Prevalence, risk factors and screening for depression: A critical review. *Acta Oncol.* **2017**, *56*, 146–55.

122. Young, K.; Singh, G. Biological Mechanisms of Cancer-Induced Depression. *Front. Psychiatry* **2018**, *9*, 299.

123. Smith, H.R. Depression in cancer patients: Pathogenesis, implications and treatment (Review). *Oncol. Lett.* **2015**, *9*, 1509–1514.

124. Pan, Y.; Zhou, F.; He, C.; Hui, L.; Huang, T.; Wei, Y. Leptin-LepRb Expressed in Gastric Cancer Patients and Related to Cancer-Related Depression. *Biomed Res. Int.* **2017**, *2017*, 1–7.

125. Suzuki, S.; Akechi, T.; Kobayashi, M.; Taniguchi, K.; Goto, K.; Sasaki, S. Daily omega-3 fatty acid intake and depression in Japanese patients with newly diagnosed lung cancer. *Br. J. Cancer* **2004**, *90*, 787–793.

126. Kobayakawa, M.; Yamawaki, S.; Hamazaki, K.; Akechi, T.; Inagaki, M.; Uchitomi, Y. Levels of omega-3 fatty acid in serum phospholipids and depression in patients with lung cancer. *Br. J. Cancer* **2005**, *93*, 1329–1333.

127. Bigornia, S.J.; Harris, W.S.; Falc, L.M.; Tucker, K.L. The Omega-3 Index Is Inversely Associated with Depressive Symptoms among Individuals with Elevated Oxidative Stress Biomarkers 1–3. *J. Nutr.* **2016**, *146*, 758–766.

128. Müller, C.P.; Reichel, M.; Mühle, C.; Rhein, C.; Gulbins, E.; Kornhuber, J. Brain membrane lipids in major depression and anxiety disorders. *Biochim. Biophys. Acta Mol. Cell Biol. Lipids* **2015**, *1851*, 1052–1065, doi:10.1016/j.bbalip.2014.12.014.

129. Smith, D.J.; Sarris, J.; Dowling, N.; Connor, M.O.; Chee, H.; Smith, D.J. Adjunctive low-dose docosahexaenoic acid (DHA) for major depression: An open-label pilot trial. *Nutr. Neurosci.* **2017**, *21*, 224–228, doi:10.1080/1028415X.2017.1283128.

130. Sánchez-Villegas, A.; Álvarez-Pérez, J.; Toledo, E.; Salas-Salvadó, J.; Ortega-Azorín, C.; Zomeño, M. Seafood Consumption, Omega-3 Fatty Acids Intake, and Life-time Prevalence of Depression in the PREDIMED-Plus Trial. *Nutrients* **2018**, *10*, 2000.

131. Parletta, N.; Zarnowiecki, D.; Cho, J.; Wilson, A.; Procter, N.; Gordon, A. People with schizophrenia and depression have a low omega-3 index. *Prostaglandins Leukot. Essent. Fat Acids* **2016**, *110*, 42–47, doi:10.1016/j.plefa.2016.05.007.

132. Orchard, T.S.; Kellie, M.M.G.; Devries, A.C. Clearing the fog: A review of the effects of dietary omega-3 fatty acids and added sugars on chemotherapy-induced cognitive deficits. *Breast Cancer Res. Treat.* **2017**, *161*, 391–398.

133. Dang, R.; Zhou, X.; Tang, M.; Xu, P.; Gong, X. Fish oil supplementation attenuates neuroinflammation and alleviates depressive-like behavior in rats submitted to repeated lipopolysaccharide. *Eur. J. Nutr.* **2018**, *57*, 893–906.

134. Kang, J.X. Fat-1 transgenic mice: A new model for omega-3 research. *Prostaglandins Leukot. Essent. Fat Acids* **2007**, *77*, 263–267.

135. Gu, M.; Li, Y.; Tang, H.; Zhang, C.; Li, W.; Zhang, Y. Endogenous Omega (n)-3 Fatty Acids in Fat-1 Mice Attenuated Depression-Like Behavior, Imbalance between Microglial M1 and M2 Phenotypes, and Dysfunction of Neurotrophins Induced by Lipopolysaccharide Administration. *Nutrients* **2018**, *10*, 1351.

136. Larrieu, T.; Layé, S. Food for mood: Relevance of nutritional omega-3 fatty acids for depression and anxiety. *Front. Physiol.* **2018**, *9*, 1047.

137. Nishinaka, T.; Yamashita, T.; Nakamoto, K.; Kasuya, F.; Tokuyama, S. Involvement of the Long-Chain Fatty Acid Receptor GPR40 in Depression-Related Behavior. *J. Pharmacol. Sci.* **2014**, *115*, 112–115.

138. Aizawa, F.; Nishinaka, T.; Yamashita, T.; Nakamoto, K.; Kurihara, T.; Hirasawa, A. GPR40/FFAR1 deficient mice increase noradrenaline levels in the brain and exhibit abnormal behavior. *J. Pharmacol. Sci.* **2016**, *132*, 249–254, doi:10.1016/j.jphs.2016.09.007.

139. Aizawa, F.; Ogaki, Y.; Kyoya, N.; Nishinaka, T.; Nakamoto, K.; Kurihara, T. The Deletion of GPR40/FFAR1 Signaling Damages Maternal Care and Emotional Function in Female Mice. *Biol. Pharm. Bull.* **2017**, *40*, 1255–1259.

140. Deyama, S.; Ishikawa, Y.; Yoshikawa, K.; Ide, S.; Satoh, M. Resolvin D1 and D2 Reverse Lipopolysaccharide-Induced Depression-Like Behaviors Through the mTORC1 Signaling Pathway. *Int. J. Neuropsychopharmacol.* **2017**, *20*, 575–584.

141. Deyama, S.; Shimoda, K.; Ikeda, H.; Fukuda, H.; Shuto, S. Resolvin E3 attenuates lipopolysaccharide-induced depression-like behavior in mice. *J. Pharmacol. Sci.* **2018**, *138*, 86–88, doi:10.1016/j.jphs.2018.09.006.

142. Ishikawa, Y.; Deyama, S.; Shimoda, K.; Yoshikawa, K.; Ide, S. Rapid and sustained antidepressant effects of resolvin D1 and D2 in a chronic unpredictable stress model. *Behav. Brain Res.* **2017**, *332*, 233–236, doi:10.1016/j.bbr.2017.06.010.

143. Chhetry, B.; Hezghia, A.; Miller, J.; Lee, S.; Rubin-Falcone, H.; Cooper, T. Omega-3 polyunsaturated fatty acid supplementation and white matter changes in major depression. *J. Psychiatr. Res.* **2016**, *75*, 65–74, doi:10.1016/j.jpsychires.2015.12.007.

144. Wu, Y.-Q.; Dang, R.-L.; Tag, M.-M.; Cai, H.-L.; Li, H.-D.; Liao, D.-H. Long Chain Omega-3 Polyunsaturated Fatty Acid Supplementation Alleviates Doxorubicin-Induced Depressive-Like

Behaviors and Neurotoxicity in Rats: Involvement of Oxidative Stress and Neuroinflammation. *Nutrients* **2016**, *8*, 243.

145. Dimitriadis, G.K.; Angelousi, A.; Weickert, M.O.; Randeva, H.S. Paraneoplastic endocrine syndromes. *Endocr. Relat Cancer* **2017**, *24*, 173–90.
146. Kir, S.; White, J.P.; Kleiner, S.; Kazak, L.; Cohen, P.; Baracos, V.E. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. *Nature* **2014**, *513*, 100–104, doi:10.1038/nature13528.
147. Azuma, M.; Gomes-Filho, J.; Ervolino, E.; Pipa, C.; Cardoso, C.; Andrada, A.C. Omega 3 Fatty Acids Reduce Bone Resorption While Promoting Bone Generation in Rat. *J. Endod.* **2017**, *43*, 970–976.
148. Tartibian, B.; Maleki, B.H.; Abbasi, A. The calciotropic hormone response to omega-3 supplementation during long-term weight-bearing exercise training in post menopausal women. *J. Sport Sci. Med.* **2010**, *9*, 245–252.
149. Graus, F.; Dalmau, J. Paraneoplastic neurological syndromes. *Curr. Opin. Neurol.* **2012**, *25*, 795–801.
150. Sioka, C.; Fotopoulos, A. Paraneoplastic immune-mediated neurological effects of systemic cancers. *Expert Rev. Clin. Immunol.* **2014**, *10*, 621–630.
151. Chen, X.; Wu, S.; Chen, C.; Xie, B.; Fang, Z.; Hu, W. Omega-3 polyunsaturated fatty acid supplementation attenuates microglial-induced inflammation by inhibiting the HMGB1/TLR4/NF-κB pathway following experimental traumatic brain injury. *J. Neuroinflamm.* **2017**, *14*, 143.
152. Obrosov, A.; Coppey, L.J.; Shevalye, H.; Yorek, M.A. Effect of Fish Oil vs. Resolvin D1, E1, Methyl Esters of Resolvins D1 or D2 on Diabetic Peripheral Neuropathy. *J. Neurol. Neurophysiol.* **2018**, *8*, 453.
153. Yorek, M.S.; Coppey, L.J.; Shevalye, H.; Obrosov, A.; Kardon, R.H.; Yorek, M.A. Effect of Treatment with Salsalate, Menhaden Oil, Combination of Salsalate and Menhaden Oil, or Resolvin D1 of C57Bl/6J Type 1 Diabetic Mouse on Neuropathic Endpoints. *J. Nutr. Metab.* **2016**, *2016*, 5905891.
154. Azar, L.; Khasnis, A. Paraneoplastic rheumatologic syndromes. *Curr. Opin. Rheumatol.* **2013**, *25*, 44–49.
155. Manger, B.; Schett, G. Paraneoplastic syndromes in rheumatology. *Nat. Rev. Rheumatol.* **2014**, *10*, 662–670, doi:10.1038/nrrheum.2014.138.

156. Barrouin-melo, S.M.; Anturaniemi, J.; Sankari, S.; Griinari, M.; Atroshi, F. Evaluating oxidative stress, serological-and haematological status of dogs suffering from osteoarthritis, after supplementing their diet with fish or corn oil. *Lipids Health Dis.* **2016**, *15*, 139, doi:10.1186/s12944-016-0304-6.

157. Dawczynski, C.; Dittrich, M.; Neumann, T.; Goetze, K.; Welzel, A.; Oelzner, P. Docosahexaenoic acid in the treatment of rheumatoid arthritis: A double-blind, placebo-controlled, randomized cross-over study with microalgae vs. sunflower oil. *Clin. Nutr.* **2018**, *37*, 494–504.

158. Capel, F.; Acquaviva, C.; Pitois, E.; Laillet, B.; Rigaudière, J.; Jouve, C. DHA at nutritional doses restores insulin sensitivity in skeletal muscle by preventing lipotoxicity and inflammation. *J. Nutr. Biochem.* **2015**, *26*, 949–959, doi:10.1016/j.jnutbio.2015.04.003.

159. Minisola, S.; Peacock, M.; Fukumoto, S.; Cipriani, C.; Pepe, J.; Tella, S.H. Tumour-induced osteomalacia. *Nat. Rev. Dis. Prim.* **2017**, *3*, 17044, doi:10.1038/nrdp.2017.44.

160. Baia, L.C.; Van den Berg, E.; Vervloet, M. G.; Heilberg I. P.; Navis G.; Bakker S. J. L.; Geleijnse J. M., Kromhout D., Soedamah-Muthu., S.S.; De Borst, M. H. Fish and omega-3 fatty acid intake in relation to circulating fibroblast growth factor 23 levels in renal transplant recipient. *Nutr Metab Cardiovasc Dis* **2014 24**, 1310-1316, doi:10.1016/ jnumecd.2014.06.006

161. Buggiani, G.; Krysenka, A.; Grazzini, M.; Vasku, V.; Hercogova, J.; Lotti, T. Paraneoplastic vasculitis and paraneoplastic vascular syndromes. *Dermatol. Ther.* **2010**, *23*,597–605.

162. Huang, T.; Wang, P.; Yang, S. Cosmetic and Therapeutic Applications of Fish Oil's Fatty Acids on the Skin. *Mar. Drugs* **2018**, *16*, 256.

163. McDaniel, J.C.; Belury, M.; Ahijevych, K.; Blakely, W. Omega-3 fatty acids effect on wound healing. *Wound Repair Regen.* **2008**, *16*, 337–345.

164. Miyata, J.; Arita, M. Role of omega-3 fatty acids and their metabolites in asthma and allergic diseases. *Allergol. Int.* **2015**, *64*, 27–34, doi:10.1016/j.alit.2014.08.003.

165. Mullard, A. 2018 FDA drug approvals. *Nat. Rev. Drug Discov.* **2019**, *18*, 85–89.

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Capítulo 3

Manuscrito do trabalho experimental

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Targeting FFA1 and FFA4 receptors as a promising approach in cancer-induced cachexia: preclinical evidence

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Abstract

Background: Omega-3 fatty acids are part of adjuvant therapy for cancer cachexia, although their mechanisms of action are unclear. Recently, the free fatty acid (FFA) receptors FFA1 and FFA4 have been demonstrated as omega-3 molecular targets in metabolic diseases. So far, their role in cancer cachexia remains unravelled. Herein, we assessed the effects of FFA1/FFA4 modulation in a mouse model of cancer cachexia.

Methods: Cancer cachexia was induced by Lewis lung carcinoma (LLC; 5 x 106) cells injection in the right flank of male C57BL/6JUnib mice (8-10 weeks old). Tumour-free mice served as negative controls. The naturally-occurring ligands α-linolenic acid (ALA) and docosahexaenoic acid (DHA), the synthetic dual FFA1/FFA4 agonists GW9508 and TUG891, or the selective FFA1 GW1100 or FFA4 AH7614 antagonists, were administered parenterally to LLC-bearing mice (7 to 21 days). Clinical, inflammatory, morphological, behavioural, and central parameters associated with LLC-cachexia were evaluated.

Results: LLC injection induced an overall body weight loss, accompanied by splenomegaly, anaemia, and an elevated neutrophil-lymphocyte ratio (NLR). ALA and DHA recovered body weight and anaemia, whereas GW9508 and AH7614 administration restored splenomegaly. Regarding tumour weight, GW9508 and TUG891 diminished this parameter. LLC-hosts displayed muscle and fat weight loss, accompanied by morphological changes. ALA restored the muscle mass, but it failed to intervene in morphological impairments. The administration of ALA, GW9508, GW1100, and AH7614 restored adipose tissue in LLC-hosts. Of note, GW9508-treated LLC-mice displayed increased serum leptin levels, while leptin epididymal fat (epWAT) was unaltered. However, GW9508 failed to rescue the LLC-induced UCP-1 downregulation in epWAT. Cachectic mice displayed deficits in locomotor activity, grip strength, and motor coordination. GW9508-treated mice displayed improved locomotor activity, while DHA treatment restored grip strength, and ALA administration improved the motor coordination. LLC-hosts displayed brain hypometabolism, which was recovered by GW9508 treatment. LLC-bearing mice displayed FFA1 upregulation in subcutaneous fat and gastrocnemius, while FFA4 was unaltered in fat depots from LLC-mice. Nevertheless, pancreatic FFA1 expression was unaltered after cachexia induction.

Conclusions: Our data shed new lights on the role of omega-3 and FFA1/FFA4 receptors in metabolic disorders, showing for the first time their relevance as pharmacological targets in cancer-related cachexia.

Keywords: omega-3, FFA1, GPR40, FFA4, GPR120, cancer cachexia

Introduction

According to the World Health Organisation (WHO), cancer is the second leading cause of death worldwide, with an estimation of 9.6-million deaths for 2018 (170). Importantly, up to 80% of these patients develop cancer-associated cachexia. At least 22% of these patients die as a result of this complication (171). Cancer cachexia is termed as a "multifactorial syndrome defined by an ongoing loss of skeletal muscle mass (with or without loss of fat mass) that cannot be fully reversed by conventional nutritional support and leads to progressive functional impairment" (70). Noteworthy, cancer-associated cachexia is still considered an unmet medical need due to the lack of pharmacological treatments (172).

The pathophysiology of cancer cachexia involves an intricate interaction between tumour and host cells. As a consequence, the systemic inflammation induced by both parties is the primary mechanism for the development of cachexia, leading to high resting energy expenditure, increased muscle atrophy, fat loss, and inappetence (75,173). Additionally, tumour-derived factors display actions similar to cytokines and contribute to the catabolic state, observed in cachexia (147,174,175). As a result, this plethora of catabolic factors leads to malnourishment, which jeopardizes the responsiveness to cancer treatment (171). Finally, these patients often display a low quality of life because of the independence loss (91).

Omega-3 fatty acids are considered beneficial for health due to its anti-inflammatory and pro-resolution characteristics (176,177). Omega-3 fatty acids have been widely used for management of cancer cachexia; however, this remains a questionable practice (17,78,169). More recently, a class of G protein-coupled receptors was identified as pharmacological targets for long-chain polyunsaturated fatty acids, such as α-linolenic acid (ALA), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (20,21). The modulation of free fatty acid (FFA) receptors, FFA1 and FFA4, previously known as GPR40 and GPR120, respectively, demonstrated favourable effects in the management of obesity, diabetes, pain, and other diseases associated to the central nervous system, such as depression, Alzheimer's and epilepsy (41,45,49,52,57,59,178–182). Furthermore, these receptors display anti-inflammatory properties, and their expression can be induced by high contents of dietary omega-3 fatty acids (41,181–184). The FFA1 and

FFA4 receptors are expressed in pancreas, brain, macrophages and adipose tissue, suggesting that they play an essential role in the metabolism regulation (20,21,50,58,185). As for the role of these receptors in cancer, the effects of their activation and/or blockage can differ depending on the tumour type (186). To our knowledge, there is no previous evidence demonstrating the effects of FFA1 and FFA4 modulation in cancer-related cachexia.

Taking into account the abovementioned evidence, the present study explored the effects of the repeated treatment with naturally-occurring and synthetic ligands of FFA1 and FFA4 receptors in a mouse model of cachexia-associated to lung carcinoma. The effects of FFA1 and FFA4 agonists and antagonists were tested on clinical, inflammatory, morphological, behavioural and central changes secondary to cachexia induction. Attempts have also been made to analyse the differential expression of FFA1 and FFA4 receptors in target tissues related to cachexia progression.

Methods

Animals

All of the experimental protocols followed the current Brazilian guidelines for the care and use of animals for scientific and didactic procedures, from the National Council for the Control of Animal Experimentation (CONCEA, Brazil, 2014), and were approved by the local Animal Ethics Committee (CEUA/PUCRS 15/7164). The report of animal studies complies with the ARRIVE guidelines (187).

Male C57BL/6JUnib specific-pathogen-free mice (20-25g; 8-10 weeks old; N=432) were obtained from the Centre for Experimental Biological Models (CeMBE/PUCRS). The N per group is indicated within the figures. All of the procedures were replicated from two to four times, elucidating the N variation among the experimental groups, due to the time elapsed for behavioural evaluations. In addition, an effort had been made to maintain the same number of animals for control groups in every assessment, to avoid losses induced by tumour implantation that could jeopardize the statistical significance. Animals were allocated to each experimental group considering their body weight before the tumour implantation. Behavioural tasks were conducted in a blinded manner, and each mouse was assessed in the behavioural tests in the

following order: mouse 1 from cage 1, mouse 1 from cage 2, mouse 1 from cage 3, and mouse 1 from cage 4, and so on.

Animals were housed in microisolator cages (3-5 per cage), equipped with inlet/outlet air filters, under controlled temperature (22 ± 1°C) and humidity (50%-70%), under a light-dark cycle of 12 hours (light on at 7 AM, lights off at 7 PM). The cages were covered with autoclaved wood chip bedding, and mice received standard pelleted chow and filtered water ad libitum. During the experimental procedures, the laboratory temperature was kept at the same as animal housing. A 30-min habituation period was allowed before the behavioural experiments and tumour implantation.

Drugs

α-linolenic acid (ALA) was purchased from Sigma-Aldrich Chemical Company (St Louis, MO, USA), and docosahexaenoic acid (DHA), GW9508, GW1100, TUG891, and AH7614 were purchased from Cayman Chemicals (Michigan, USA). ALA, DHA, GW9508, and TUG891 were diluted in ethanol and stored at -20°C. GW110 and AH7614 were diluted in dimethyl sulfoxide (DMSO) and stored at -20°C. All compounds were prepared at the desired concentration using phosphate-buffered saline (PBS) as vehicle, prior to treatments, maintaining DMSO or ethanol final concentrations under 2%.

Despite the dual agonism of GW9508 for FFA1 and FFA4 receptors, the affinity for FFA1 is 100-fold higher, in comparison to FFA4 (29). Similarly, TUG891 is also considered a dual agonist for both receptors in mice; however, its selectivity is 61-fold higher (β-arrestin-2 assay), and 3-fold higher for FFA4, in relation to FFA1 (Ca+ mobilization assay) (188). Regarding the antagonists used in this study, GW1100 and AH7614 are both selective antagonists for FFA1 and FFA4, respectively (29,189).

Lewis lung carcinoma cell line culture and tumour implantation

The Lewis lung carcinoma cell line (LLC; BCRJ 0145, Rio de Janeiro, Brazil) was cultured in 150-mL culture flasks in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% foetal bovine serum plus 1% penicillin and streptomycin. After reaching confluence, cells were trypsinized, counted using a Neubauer chamber, centrifuged (10 min in 3000 RPM, at 25°C), and resuspended for implantation. LLC cells were used for the induction of the cancer cachexia model, after 3 to 4 passages.

For this purpose, LLC cells (5 x 106) were resuspended in 100 µl of PBS and injected subcutaneously (s.c.) in the right hind flank of previously anesthetized mice (10 mg/kg xylazine + 100 mg/kg ketamine) (147). Tumour-free controls had been also anesthetized and received PBS (s.c.) at the same volume and site of injection as the tumour-injected mice. Twenty-one days after tumour cell implantation, mice were euthanized by sevoflurane inhalation. Afterwards, total blood was collected from the abdominal aorta. The tumour mass was isolated for tumour-free carcass weight assessment, and the following tissues were weighed (in g), fixed in formaldehyde and/or frozen in -80°C: gastrocnemius, tibialis anterior and soleus muscles, retroperitoneal (rWAT), epididymal (epWAT) and intrascapular (isWAT) adipose tissues, and spleen.

Treatment schemes

Firstly, to assess the effects of naturally occurring omega-3 fatty acids in the mouse model of cancer-induced cachexia, ALA and DHA were administered intraperitoneally (i.p.). ALA (2.5 and 5 mg/kg) was administered every other day, whereas DHA (1 µmol/kg) was dosed daily, from day 7 to euthanasia. Next, the relevance of FFA1 and FFA4 receptors in cancer cachexia was investigated by treating animals with the dual FFA1/FFA4 agonists GW9508 (2 and 8 mg/kg) and TUG891 (2 and 8 mg/kg). Separate experimental groups were treated with the selective FFA1 or FFA4 antagonists, GW1100 (4 and 10 mg/kg) and AH7614 (4 and 10 mg/kg), respectively. The synthetic ligands were administered s.c., every other day, from day 7 to euthanasia. Tumour-free control animals received PBS i.p. or s.c, at the same time points.

The doses and protocols of treatment with ALA, DHA and GW9508 were chosen based on previous publications (41,190,191), with slight modifications. To select the doses of GW1100, TUG891 and AH7614, we used the first dose of GW9508 (2 mg/kg) as a reference for the calculations of the following doses (8 mg/kg, 4 mg/kg, and 10 mg/kg, respectively), based on the EC50 of each molecule (20–22,29). Figure 1 summarises the general experimental procedures and treatment schemes. All of the compounds were tested on clinical, behavioural and haematological changes associated to cachexia. The most effective doses and compounds were further evaluated in histochemical, biochemical, molecular biology and imaginological assays.

Behavioural assessments

Spontaneous locomotor activity

To evaluate the spontaneous locomotor activity of tumour-bearing mice, behavioural assessments were carried out, as previously described (192). For this, an automatic open-field system comprised of an acrylic box (46 x 46 x 36 cm) with infrared sensors was used. Firstly, mice were adapted in the behavioural test room, for at least 30 min. Subsequently, mice were placed in the centre of the open-field apparatus, with 1 min for habituation and 5 min for locomotor activity analysis. The activities registered were ambulatory movement, rearing, travelled distance (cm) and speed (mm/s). Movements were monitored on the x, y, and z-axes, which represent the height, width, and depth, respectively.

Rota-rod activity

The motor coordination and balance maintenance were evaluated in the rota-rod apparatus, as previously described by Silva et al. (192), 30 min after the spontaneous locomotor activity evaluation. The rota-rod apparatus consisted of a rod (3-cm in diameter) with five flanges, allowing the simultaneous evaluation of four mice. Mice were placed in the device at a speed of 16 RPM until 60 s. To assess motor coordination deficiencies, the latency to fall was recorded in seconds. Mice were previously trained in the device, before tumour implantation.

Grasping test

With the intent to assess the strength of mice after cachexia induction, the grasping test was performed 30 min after the rota-rod evaluation, as formerly described, with slight modifications (193,194). After a gentle lifting by the tail, mice were allowed to grasp a grid positioned on an electronic balance. Animals were submitted to three trials, each one with a 10-second interval. The number (in grams) showed by the balance was instantly recorded, and a mean was calculated after the three trials to calculate the individual grasping strength.

Haematological parameters

Immediately post-euthanasia, the whole blood was collected from the abdominal aorta, and a small drop of blood was used for the smear evaluation, using Giemsa staining. Differential cell counting (neutrophils, lymphocytes, eosinophils, basophils, monocytes, and immature cells) was performed using an x100 objective, by counting 100 cells. Then, the neutrophil-lymphocyte ratio was calculated as an indicatory of systemic inflammation. A part of the whole blood was used for the haematocrit analysis. Blood samples were placed in heparin tubes, and a small drop was collected using a capillary tube. Next, the capillary tubes filled with blood were centrifuged in a cytocentrifuge, for 5 min, 8000-x g. The haematocrit value was calculated as the percentage of cellular sedimentation by total blood volume.

Histological analysis

Gastrocnemius muscle, and subcutaneous and visceral adipose tissues were removed following euthanasia, at the 21st day after tumour implantation. Tissues were fixed in 10-% formaldehyde, during 24 h. Subsequently, tissues were included in paraffin and sectioned in 5-µm slices, for staining with haematoxylin and eosin. Images were captured using a Zeiss AxioImager M2 light microscope, under 200 x magnification (Carl Zeiss, Gottingen, Germany) and were analysed using NIH ImageJ 1.36b Software. The area of each adipocyte was measured manually, using the free-hand function, after adjusting the image scale in µm2. The tissues analysed were selected based on the statistical difference of the tissue weight between the LLC control group and LLC-treated mice.

Immunohistochemistry for FFA1 and FFA4 receptors

With the intent to further evaluating the participation of free fatty acid receptors in adipose tissue modulation after cachexia induction, the distribution of FFA1 and FFA4 was evaluated by immunohistochemistry, as described previously (41). Adipose tissues (isWAT and epWAT) from LLC-injected and tumour-free control mice were collected after euthanasia, and fixed in 10-% buffered formalin solution, for 24 h. The immunopositivity for FFA1 and FFA4 was assessed in paraffin-embedded tissue sections (4-µm thickness), using anti-FFA1 (1:100; item no. 10007205; Cayman Chemicals, Michigan, USA) and anti-FFA4 (1:500; Catalog no. A19859, Boster Bio, Pleasanton, USA). Images were captured by Zeiss AxioImager M2 light microscope in 200-x magnification. In order to evaluate the FFA1 and FFA4

immunopositivity, images were transferred to a computer and were analysed in NIH ImageJ 1.36 Software. For this, a specific macro was created using dark-and-brown pixels as reference of positive areas. This macro was applied in all images from both experimental groups and the results were expressed in mean grey values.

Western blotting

To evaluate the development of thermogenesis associated to cachexia, the expression of UCP-1 (uncoupling protein-1) was analysed in the epWAT of LLC-injected mice treated with GW9508 (8 mg/kg, s.c.), and the respective controls. epWAT samples from the respective controls were also included in the analysis. Also, the expression of FFA1 was evaluated in the pancreas and gastrocnemius from LLC-bearing mice treated with GW9508 (8 mg/kg, s.c.), and their respective controls. Tissues were isolated after euthanasia and stored in an ultra-freezer at -80°C. The western blotting protocol was performed as described previously, by Amaral et al. (195), with slight modifications for protein extraction. Tissues were homogenised using RIPA buffer, according to the manufacturer's instructions. The protein concentration was determined using the Coomassie Protein Kit, as described by the manufacturer. The membranes were incubated overnight with the primary antibodies anti-UCP-1 (1:1000), anti-FFA1/GPR40 (1:500) or anti-β-actin (1:20.000). The samples were incubated for an additional period of 2 h with the anti-rabbit or anti-mouse secondary antibodies. The Novex® ECL and the KODAK Gel Logic 2200 Imaging System were used for detecting the protein bands. Quantification of each band was made using Image J 1.36b Software (NIH, Bethesda, MD, USA). Data from western blot experiments is expressed in arbitrary units, calculated in relation to the expression of β-actin of the corresponding sample.

Leptin and cytokines assessment

Serum, gastrocnemius, rWAT and epWAT were collected from LLC-injected mice treated with GW9508 (8 mg/kg, s.c.), and from the respective controls, to evaluate the levels of tumour necrosis factor (TNF), interleukin (IL)-1β, IL-10, interferon (IFN)-γ and leptin. These cytokines were assessed by sandwich enzyme-linked immunosorbent assay using DuoSet kits according to the manufacturer's

instructions (R&D Systems, Minneapolis, MN). The results are expressed in pg/mL or pg/mg for serum and tissue, respectively.

MicroPET imaging

Brain glucose metabolism is altered in cachectic mice (196). This set of experiments was accomplished as formerly described (197), aiming at evaluating the cerebral alterations associated with cancer-induced cachexia and the effects of treatment with omega-3-related ligands (192,193). LLC-injected mice treated with ALA (2.5 mg/kg, i.p.), DHA (1 µmol/kg, i.p.), or GW9508 (8 mg/kg, s.c.) were scanned on the 21st day after tumour injection, and euthanised. For the scanning in Triumph microPET (LabPET-4; TriFoil Imaging, Northridge, CA), each mouse was anesthetised with isoflurane and medical oxygen (3%-4% induction and 2%-3% maintenance dose) and [18F]-FDG was injected (250 µCi) through the tail vein. After 40 min of conscious uptake, mice were placed in a head prone position in the imaging chamber and maintained at a constant temperature of 36°C, under isoflurane inhalation. Animals were scanned during 10 min, with the brain positioned in the centre of the microPET field-of-view. Images were reconstructed using the algorithm MLEM-3D. The microPET images were spatially normalised into an [18F]-FDG template using brain normalization in PMOD v3.8 and the Fuse It Tool (PFUSEIT) (PMOD Technologies, Zurich, Switzerland). The glucose metabolism was expressed as standard uptake values (SUVs) for several brain regions.

Biodistribution assay

In order to evaluate the [18F]-FDG biodistribution ex vivo, mice were euthanised immediately after the microPET image acquisition. Subsequently, the brain and tumour mass were removed, placed in previously weighted tubes, and their radioactivity was measured in a γ-counter (2480 WIZARD2 automatic gamma counter, PerkinElmer, Waltham, MA, USA). The radioactivity uptake in the tumour and brain was expressed as the percentage of radioactivity injected per gram of tissue (%ID/g) (198).

Statistical analysis

Results were expressed as the mean ± SEM. The Bartlett test was used to check data normality. Statistical analysis was performed by Kruskal-Wallis (non-parametric data) or one-way analysis of variance (one-way ANOVA) (parametric data) for comparison of tumour-free control vs LLC-injected mice, with or without treatments. When the group interactions were statistically significant ($p < 0.05$), pairwise comparisons were carried out using Dunn or Bonferroni post-hoc tests, after Kruskal-Wallis or one-way ANOVA, respectively. Statistical tests and design of graphs were held using GraphPad Software version 8.1.1.0 (GraphPad Software Inc. San Diego, CA, USA).

Results

FFA1 and FFA4 ligands modulate clinical features of cachexia and tumour growth

The induction of cancer cachexia was investigated by the s.c. implantation of LLC cells into the right hind flank of mice. As expected, the implantation of tumour cells generated a loss of the whole-body weight and led to skeletal muscle atrophy and fat loss, according to the assessment after 21 days of tumour growth (Figures 2A-B, 3A-B, 4A-B, Tables 1,2 and 3). To evaluate the role of FFA1 and FFA4 receptors in cancer-associated cachexia, we initially assessed the effects of naturally occurring agonists in the LLC-cachexia model. Regardless of the pharmacological promiscuity of omega-3 PUFAs, we selected ALA and DHA as FFA1/FFA4 naturally-occurring agonists, based on previous publications (22,41,181,199). The induction of cachexia by LLC cells impaired tumour-free carcass weight in a significant manner, as evaluated after euthanasia and tumour mass removal (Figure 2A; $P<0.05$). Regarding the treatment with omega-3 PUFAs, the systemic administration of both doses of ALA (2.5 and 5 mg/kg, i.p.) significantly restored the tumour-free carcass weight to control values (Figure 2A; $P<0.05$). On the contrary, the systemic treatment with DHA (1 μmol/kg; i.p.) failed to reverse LLC-induced decrease of tumour-free carcass weight (Figure 2A). Relating to the treatment with the synthetic ligands of FFAs receptors, the dual FFA1/FFA4 agonist GW9508 (8 mg/kg, s.c.) and the FFA1 selective antagonist GW1100 (4 and 10 mg/kg, s.c.) rescued this feature to tumour-free control values (Figure 3A). Alternatively, the repeated treatment with the dual FFA1/FFA4 agonist TUG891 (2 and 8 mg/kg, s.c.) or FFA4 selective antagonist AH7614 (4 and 10 mg/kg, s.c.) failed to recover the tumour-free carcass weight loss (Figure 4A).

Concerning the body weight variation (which comprises of the difference between the whole-body weight at the time of LLC-cell implantation, and the tumour-free carcass weight at the euthanasia), tumour-

bearing mice displayed a marked weight loss throughout the 21 days of experiment (Figure 2B, 3B, and 4B; $P<0.05$). Regarding ALA (2.5 mg/kg, i.p.) administration, body weight variation was recovered by this treatment in a significant manner (Figure 2B; $P<0.05$), while the higher dosage of ALA (5 mg/kg, i.p.) failed to reverse the weight loss. In addition, the daily DHA treatment (1 μmol/kg, i.p.) significantly maintained the body weight gain during the 21-day experimental period, similar to tumour-free control animals (Figure 2B; $P<0.05$). On the contrary, the repeated administration of the synthetic ligands of either FFA1 or FFA4 did not alter the ongoing weight loss in cachectic mice (Figure 3B, 4B; $P>0.05$).

The tumour mass was allowed to grow during the 21 days after LLC cells implantation, reaching a mean value of 2.41±0.17 g (Figure 2C, 3C, 4C). Concerning the treatment with ALA (2.5 and 5 mg/kg, i.p.) and DHA (1 μmol/kg, i.p.), there was no statistical difference among the experimental groups (Figure 2C). Notably, the dual FFA1/FFA4 agonist GW9508 (8 mg/kg, s.c.) significantly reduced the tumour weight, and a comparable reduction of tumour mass was observed after the repeated treatment with the also mixed FFA1/FFA4 agonist TUG891 (2 mg/kg s.c.) (Figure 3C, 4C; $P<0.05$). The selective antagonists for FFA1 and FFA4, GW1100 and AH7614, respectively, failed to reduce the tumour weight (Figure 3C, 4C).

Modulation of FFA1 and FFA4 receptors and cachexia-related inflammation

The spleen weight was evaluated as an indicative of the inflammatory state. Accordingly, LLC-injected groups demonstrated a marked splenomegaly (Figure 2D, 3D, 4D; $P<0.05$). The treatments with the omega-3 PUFAs ALA (2.5 and 5 mg/kg, i.p.) or DHA (1 μmol/kg, i.p.) failed to reverse this aspect (Figure 2D). Of note, the dual FFA1/FFA4 agonist GW9508 (8 mg/kg, s.c.) or the selective FFA4 antagonist AH7614 (4 mg/kg, s.c.) recovered the splenomegaly in LLC-bearing mice, towards tumour-free control levels (Figure 3D, 4D). However, the FFA1 antagonist GW1100 (4 and 10 mg/kg, s.c.) or the dual FFA1/FFA4 agonist TUG891 (2 and 8 mg/kg, s.c.) failed to rescue the splenomegaly associated to cancer progression (Figure 3D, 4D; $P>0.05$).

LLC-bearing mice displayed severe anaemia, as shown by a significant reduction of haematocrit values (Figure 2E, 3E, 4E; $P<0.05$). Remarkably, the systemic treatment with ALA (2.5 mg/kg, i.p.) significantly recovered this state (Figure 2E; $P<0.05$). A similar, but not significant effect was observed for the higher dose of ALA (5 mg/kg, i.p.) (Fig 2E) Additionally, the DHA (1 μmol/kg, i.p.) treatment partially

recovered cachexia-associated anaemia, when compared to LLC-bearing mice (Figure 2E; P=0.09). Alternatively, the FFAs synthetic ligands failed to revert cancer-associated anaemia (Figure 3E and 4E).

The neutrophil-lymphocyte ratio (NLR) is a prognosis factor related to cancer survival (200). As expected, LLC-cachectic mice showed a marked increase of NLR (Figure 2F, 3F, 4F; $P<0.05$), whereas FFA1 and FFA4 naturally-occurring or synthetic ligands lacked any effect on this cachexia-related feature.

Leptin and cytokines assessment on LLC-bearing mice

To further characterize the inflammatory changes in cachectic mice, the levels of several cytokines were assessed. Serum leptin levels in LLC-injected mice did not significantly differ from tumour-free control mice, whereas LLC-bearing mice treated with GW9508 (8 mg/kg, s.c.) exhibited a marked increase of circulating leptin contents (Figure 5A; $P<0.05$). Conversely, there was no significant difference in the epWAT leptin levels, when comparing tumour-free control and LLC-bearing mice, irrespective of treatment with GW9508 (5B). Regarding the analysis of TNF, IL-1β, IFN-γ and IL-10 in serum, gastrocnemius, rWAT and epWAT, the levels of the referred cytokines were under the limit of detection (results not shown).

Effects of FFA1 and FFA4 ligands in muscle wasting and fat loss

The induction of cachexia led to an important overall tissue weight reduction, as it can be seen in Tables 1, 2 and 3. Skeletal muscles suffered a decrease in tissue weight in LLC-injected mice, an effect that was restored by ALA treatment (2.5 and 5 mg/kg, i.p.) for the gastrocnemius muscle, in a significant manner (Table 1; $P<0.05$). Elsewise, the skeletal muscle weights were not significantly altered by the systemic treatment with the DHA (Table 1) or the synthetic ligands of FFA1 and FFA4 receptors (Table 2 and 3).

Concerning the adipose tissue remodelling, the lower dose of ALA (2.5 mg/kg, i.p.) recovered isWAT weight loss in a significant manner ($P<0.05$). In addition, DHA (1 μmol/kg, i.p.) administration recovered the weight losses of isWAT and epWAT reaching control levels, but this effect was not significant (Table 1). A recovery of isWAT, epWAT and rWAT tissue weights was also observed after the treatment with the dual FFA1/FFA4 agonist GW9508 (8 mg/kg, s.c.) (Table 2; $P<0.05$), whereas TUG891 did not elicit any significant effect on cachexia-related lipolysis (Table 3). Interestingly, the treatment with

the selective FFA1 and FFA4 antagonists, GW1100 or AH7614 (both at 4 mg/kg), respectively, similarly restored the fat loss in LLC-bearing mice (Tables 2 and 3; $P<0.05$). It is worth of mentioning that repeated treatments with ALA, DHA, GW9508, GW110, TUG891, or AH7614 in tumour-free control mice did not alter the skeletal muscle or adipose tissue weights, as depicted in Tables 1, 2, and 3.

Skeletal muscle alterations associated to LLC-associated cachexia

As previously described, the treatment with ALA (2.5 and 5 mg/kg, i.p.) was able to significantly restore the muscle wasting in cachectic mice. Thus, we performed an analysis of the cross-sectional area of muscle fibres and the number of central myonuclei in the gastrocnemius of ALA-treated groups and their respective controls. There were no significant differences in fibre size frequency distribution among the experimental groups (Figure 6A). The muscle fibres tended to display a myonuclei centralization when in degeneration in LLC-cachectic mice, regardless of ALA treatment (Figure 6B). Representative histological images of the gastrocnemius muscle in the different experimental groups are depicted in Figure 6C-F.

Histological alterations of adipocytes in LLC-cachectic mice after treatment with FFA1 and FFA4 ligands

Based on the effects of the repeated treatment with the naturally-occurring agonists and the synthetic ligands on adipose tissue weight, we further analysed the cross-sectional area of adipocytes, to estimate the adipocyte size among the experimental groups. It is important to emphasize that the following results are related to the adipose tissues that displayed statistical difference between control and treated LLC-bearing mice (depicted in Table 1, 2 and 3). Regarding the average adipocyte area in isWAT of LLC-mice treated with ALA, there was no statistical difference between experimental groups (Figure 7A). As to the adipocyte cross-sectional area frequency distribution, LLC-injected mice displayed a significant higher number of adipocytes with 300-400 μm2, compared to tumour-free control mice (Figure 7B; $P<0.05$). LLC-bearing mice treated with ALA (5 mg/kg, s.c.) showed a higher number of adipocytes with an area ranging from 600 to 700 μm2, in comparison to tumour-free control and LLC-bearing mice (Figure 7B; $P<0.05$). Notably, the treatment of LLC-bearing mice with ALA (2.5 mg/kg, i.p.) rescued the adipocyte cross-sectional areas towards tumour-free control adipocytes (Figure 7B; $P<0.05$). Representative histological images of isWAT are depicted in Figure 7C-F.

Regarding the effects of the synthetic ligands on isWAT adipocyte cross-sectional area (Figure 8), there were no statistical differences among the experimental groups (Figure 8A). The treatment with the selective FFA1 antagonist GW1100 (4 mg/kg, s.c.) led to a reduction in the frequency of adipocytes smaller than 100 μm2, in comparison to tumour-free controls and LLC-bearing mice (Figure 8B; $P<0.05$). However, the implantation of LLC cells significantly increased the number of adipocytes with a cross-sectional area of 100-200 μm2, irrespective of the treatment with GW9508 (8 mg/kg, s.c.) (Figure 8B; $P<0.05$). Representative images of isWAT from all of the experimental groups are depicted in Figures 8C-F. The average adipocyte cross-sectional area in rWAT from tumour-free control and LLC-bearing mice did not differ in a significant manner (Figure 8G). However, LLC-bearing mice treated with GW9508 (8 mg/kg, s.c.) displayed a significant decrease of average adipocyte area in comparison to tumour-free controls (Figure 8G; $P<0.05$). Regarding the adipocyte area frequency distribution, the number of adipocytes with a cross-sectional area <100 μm2 was significantly higher in LLC-bearing animals, with a decrease in the frequency of adipocytes with areas ranging from 1000-1400 μm2 (Figure 8H; $P<0.05$). LLC-bearing mice treated with GW9508 (8 mg/kg, s.c.) demonstrated a higher number of adipocytes with areas ranging from 100 to 500 μm2, when compared to tumour-free control and LLC-bearing mice (Figure 8H; $P<0.05$). Additionally, the repeated treatment with the selective FFA4 antagonist AH7614 (4 mg/kg, s.c.) significantly increased the frequency of adipocytes with areas ranging from 1000-1300 μm2 (Figure 8H; $P<0.05$). Representative images of rWAT are shown in Figure 8I-M. The average adipocyte area in epWAT from tumour free-control mice and LLC-bearing mice, apart from the treatment with GW9508 (8 mg/kg, s.c.), did not demonstrate any statistical difference (Figure 8N; $P>0.05$). However, GW9508-treated LLC-bearing mice displayed a significant increase in the number of adipocytes with areas from 700-800 μm2, accompanied by a reduction of adipocytes with areas from 1500-1600 μm2 (Figure 8O; $P<0.05$). Representative images of experimental groups are shown in Figures 8P-R.

Modulation of UCP-1 expression in white adipose tissue of cachectic mice

The UCP-1 expression has been previously evaluated as a thermogenesis marker (153). Herein, UCP-1 was analysed in epWAT of LLC-bearing mice after the treatment with GW9508 (8 mg/kg, s.c.). Tumour-free control mice displayed a constitutive expression of UCP-1 in epWAT, while LLC-bearing

mice exhibited a significant decrease of UCP-1 expression, regardless of the treatment with GW9508 (Figure 9A, $P<0.05$). The representative blot is provided in Figure 9B.

Assessment of behavioural deficits associated to LLC-cancer induced cachexia

The induction of cancer cachexia by LLC-cells implantation evoked severe alterations related to mobility and strength after 21 days of tumour growth, displayed by a diminished spontaneous locomotor activity, impaired grip strength and decreased motor coordination (Figures 10, 11 and 12). While the repeated administration of ALA (2.5 and 5 mg/kg, i.p.) failed to rescue the reduced ambulatory movement, travelled distance, rearing, and speed (Figure 10A-D), DHA (1 µmol/kg, i.p.) treatment partially recovered the ambulatory movement (Figure 10A; $P=0.10$). Concerning the synthetic ligands, the repeated administration of GW9508 (8 mg/kg, s.c.) significantly rescued the spontaneous locomotor activity (Figure 11A-D; $P<0.05$), while the administration of TUG891 (8 mg/kg, s.c.) failed to alter this feature (Figure 12A-D). The selective antagonists GW110 and AH7414 lacked any effect on the locomotor deficits associated to cancer cachexia (Figure 11A-D, 12A-D).

The repeated treatment with DHA (1 µmol/kg, i.p.) recovered the impaired grip strength in a significant manner, whereas ALA (5 mg/kg, i.p.) significantly improved the motor coordination of cachectic mice (Figure 10E and 10F, respectively; $P<0.05$). The repeated administration of GW9508 (8 mg/kg, s.c.) restored the grip strength toward the control levels (Figure 11E). In a different manner, both doses of GW1100 failed to modify all of the assessed parameters (Figure 11E and 11F). Importantly, the systemic administration of AH7614 (4 mg/kg, s.c.) significantly rescued the reduced grip strength associated to cancer cachexia (Figure 12E; $P<0.05$). In respect to the impaired motor coordination induced by LLC-cells implantation, TUG891 and AH7614 failed to recover this parameter (Figure 12F).

Brain hypometabolism in cancer-associated cachexia

In the first experimental set (Figure 13), LLC-bearing mice were scanned in the 21st day after the repeated administration of ALA (2.5 mg/kg, i.p.) or DHA (1 µmol/kg, i.p.), and compared to their respective controls. The whole brain glucose uptake did not differ among the experimental groups (Figure 13A). However, when the brain regions were analysed separately, the LLC-injected mice demonstrated an overall

reduction of cerebral glucose metabolism, irrespective of the treatment with ALA or DHA (Figure 13B and C). Representative images of [18F]-FDG uptake are depicted in Figures 13D-G.

In the second experimental set (Figure 14), LLC-injected mice and their respective controls were analysed after the systemic treatment with GW9508 (8 mg/kg, s.c.). Concerning the whole brain metabolism, the glucose uptake remained similar among the experimental groups (Figure 14A). Nevertheless, when the glucose metabolism was analysed throughout separate brain regions, LLC-bearing mice displayed a significant brain hypometabolism in right and left striatum, cortex, left hypothalamus, thalamus, superior colliculus and right inferior colliculus. Of note, the repeated treatment with GW9508 reinstated brain metabolism to control values (Figure 14B and C). Interestingly, the tumour mass and the brain radioactivity were unaltered among the different experimental groups, demonstrating that brain hypometabolism in LLC-bearing mice did not depend on the tumour mass (Figure S1). Representative images of brain [18F]-FDG uptake are shown in Figures 14D-F.

FFA1 and FFA4 immunopositivity in adipose tissue from LLC-bearing mice

An immunohistochemistry assay was carried out to evaluate the immunopositivity for FFA1 and FFA4 receptors in epWAT and isWAT of tumour-free control and LLC-injected mice. Notably, the FFA1 receptor was upregulated in isWAT of LLC-bearing mice (Figure 15A; $P<0.05$), whilst the FFA4 receptor immunopositivity was not significantly different (Figure 15D). The representative images of the immunopositivity for FFA1 and FFA4 in isWAT are shown in Figure 15B-C and 15E-F, respectively. Regarding the FFA1 and FFA4 expression in epWAT, the immunopositivity of both receptors was not significantly different, when comparing tumour-free and LLC-bearing mice (Figure 16A, 16D). Representative images of the immunopositivity for both receptors in epWAT are depicted in Figure 16B-C and 16E-F.

FFA1 protein expression in muscle and pancreas of LLC-bearing mice

We assessed the expression of FFA1 in the pancreas and gastrocnemius muscle of LLC-bearing mice treated with the dual agonist GW9508 (8 mg/kg, s.c.). Interestingly, the FFA1 protein expression in the gastrocnemius suffered a significant increase in LLC-bearing mice, and diminished approximating to

control values in GW9508-treated LLC-bearing mice (Figure 17A; $P<0.05$). The FFA1 receptor was constitutively expressed in pancreas of tumor-free control mice, without any significant variation by tumor induction or GW9508 (8 mg/kg, s.c.) treatment (Figure 17C). The representative blots for FFA1 in gastrocnemius and pancreas are depicted in Figures 17B and D, respectively.

Discussion

Cancer-associated cachexia is a multifactorial syndrome that is considered an unmet medical need. The use of omega-3 fatty acids in this condition is a common approach in clinical practice, with conflicting, but promising outcomes (17,169,201,202). Herein, we evaluated the effects of ALA and DHA in a mouse model of cancer cachexia. Additionally, in virtue of their unspecific pharmacological properties, we also assessed the impacts of FFA1 and FFA4 synthetic ligands in this cachexia animal model. The main idea was to investigate to what extent these receptors can modulate cancer cachexia and if omega-3 fatty acids benefits are linked, at some level, to the FFA1/FFA4 receptor activation. Remarkably, the dual FFA1/FFA4 agonist GW9508 displayed several beneficial effects on the LLC-cachexia model, more importantly, on adipose tissue modulation, splenomegaly-associated cancer cachexia, impaired behavioural performance, and central alterations, besides reducing the tumour mass. It is worthy of mentioning that this is the first evidence showing that cachexia induction led to changes of FFA1 expression in the adipose tissue.

In alignment with other LLC-cachexia models, we showed a decrease of tumour-free carcass weight and an impaired body weight variation throughout the experimental period of 21 days (203,204). Tumour-free carcass weight is a significant characteristic to measure body weight loss; however, it can be overestimated depending on the initial body weight (before LLC-cells implantation). The body weight variation is more useful to detect the ongoing body weight loss because of the difference between the initial body weight and tumour-free carcass weight, correcting any body weight variation among the same experimental group. Importantly, the systemic treatment with ALA and DHA recovered the ongoing body weight loss, and the FFA1 and FFA4 synthetic ligands failed to reverse this important feature. Regarding the effects of ALA in body weight variation, a study in Walker 256 tumour-bearing rats demonstrated that an ALA-enriched diet recovered the body weight loss. Nevertheless, Carnier et al. demonstrated that a diet containing chia flour failed to modulate cancer cachexia in the same animal model (190,205). These conflicting results can be explained, at some level, by the route of administration. In both studies, rats

received an ALA-enriched diet, likely decreasing the dosage absorbed. Herein, cachectic mice probably displayed a better result in virtue of the parenteral route selected for our research. Additionally, it was previously shown that a DHA-enriched diet protected ongoing weight loss in tumour-bearing rats receiving doxorubicin and in mice after 48-h fasting, corroborating our results (206,207). In virtue of the distinct results from ALA and DHA treatment and FFA1 and FFA4 synthetic ligands on body weight alterations, we can conclude that beneficial effects of ALA and DHA do not rely on the activation of FFA1/FFA4 receptors. Alternatively, they might be related to the modulation of membrane composition and inhibition of inflammatory mediators (166,208).

Noteworthy, we observed positive effects on tumour weight regarding the FFA1 and FFA4 ligands: either agonists that are considered dual for both receptors decreased tumour mass in a significant manner, whereas the selective antagonists did not alter this feature. Kita et al. (69) demonstrated that GW9508 treatment modulated the motility of LLC-cells, with opposite effects for FFA1 and FFA4 receptors. As for the impact of TUG891 in lung cancer, there is no evidence corroborating or contrasting with our result. However, it was demonstrated that the activation of FFA4 receptors by TUG891 induced resistance of hormone receptor-positive breast cancer cells to tamoxifen therapy, contrasting somewhat with our data (209). Also, the in vitro treatment with EPA, GW9508, or TUG891 failed to alter rat pituitary tumour cells viability (210). On the contrary, in human ovarian cancer cells, GW9508 treatment significantly inhibited the cell proliferation via FFA1 receptor activation (211). When analysed together, these pieces of evidence extend the previous notion that FFA1/FFA4 receptors might display contrasting effects depending on the tumour type (186).

An important feature linked to the high inflammatory state present in cachexia was the marked splenomegaly in LLC-bearing mice. Corroborating with our cachexia model, Kandarian et al. (212) demonstrated a similar splenomegaly development in C26 tumour-bearing mice. The tested naturally-occurring ligands ALA and DHA did not alter splenomegaly. However, our data showed that the dual FFA1/FFA4 agonist GW9508 rescued the splenomegaly-associated to cachexia, while the selective FFA4 antagonist AH7614 induced a similar result. This might be indicative that the prevention of splenomegaly by GW9508 is mediated by FFA1 activation. According to Gotoh et al. (213), FFA1 and FFA4 are both expressed in the spleen of mice. Nevertheless, there is no further evidence demonstrating splenomegaly reduction by activation and blockage of both FFAs receptors.

The reduced haematocrit values in cachectic mice is likely associated with splenomegaly since this condition is related to the destruction of erythrocytes (214). Indeed, the implantation of C26 colon carcinoma, B16 melanoma and LLC tumor cells in mice also led to anaemia development (215). Additionally, in the clinical scenario, a low haematocrit is considered an independent risk factor for poor diagnosis in lung cancer patients (216). In our study, the cachexia-associated anaemia was recovered by the parenteral treatment with the naturally-occurring omega-3 fatty acids ALA and DHA, with slight effects for GW9508, TUG891, and AH7614. Omega-3 fatty acids intervention failed to increase the haematocrit levels in haemodialysis patients, different from our results (217).

A high NLR is considered a marker for poor prognosis in cancer patients (200). We observed an increase of NLR in LLC-bearing mice, an effect that remained unaffected by the treatment with either natural or synthetic FFA1/FFA4 ligands. In alignment with our results, Huang et al. also demonstrated an increased rate of circulating neutrophils in LLC-cachectic mice (218). On the contrary, it was previously demonstrated that omega-3 fatty acid supplementation diminished tumour-NLR in a mouse model of metastatic breast cancer, although the authors did not evaluate the circulating NLR (219), what might account for the different results.

With the intent to expand our notion regarding the inflammatory state of LLC-cachectic mice, we analysed tissue and circulating cytokine levels. We were unable to detect TNF, IL-1β, IL-10, and IFN-γ in tissue and serum of cachectic mice. Our lack of cytokine detection can be due to our chosen experimental period of 21 days. Accordingly, Henriques et al. (203) showed altered levels of several circulating cytokines in LLC-mice at 28 days of LLC inoculation. We failed to detect significant differences when comparing the circulating leptin levels in tumour-free control and LLC-bearing mice. Corroborating our data, Tong et al. performed a meta-analysis and concluded that circulating leptin is not involved with cachexia development in lung cancer patients (104). Additionally, it was demonstrated that in chronic cachexia, circulating leptin levels are decreased (149). However, we observed an increase of circulating leptin levels in LLC-bearing mice treated with GW9508 and unaltered leptin levels in epWAT from these same animals. One might suppose that elevated serum leptin levels might reach the hypothalamus, modulating the food intake and the energy expenditure, helping to explain the multifaceted favourable effects of GW9508 in our experimental paradigm.

Cancer cachexia is mainly characterized by skeletal muscle atrophy (171). As demonstrated in the cachexia model induced by C26 colon adenocarcinoma inoculation in mice, we demonstrated decreased gastrocnemius and tibialis muscle weight (212). Herein, the systemic administration of ALA rescued the gastrocnemius weight, without any effects for the other tested ligands. Corroborating with our results, a clinical study with cachectic gastrointestinal cancer patients demonstrated that a fish oil-enriched oral supplement increased skeletal muscle mass, after six months of treatment (220). The effects of ALA do not appear to be related to the activation of FFA1 or FFA4 receptors. It is tempting to suggest that ALA might recover muscle wasting by membrane lipid modulation or by the degradation in EPA, DHA, or pro-resolution mediators. In fact, Resolvin E1 improved myotube morphology in an in vitro study of muscle atrophy induced by bacterial endotoxin, rather supporting our suggestion (221). To gain further insights regarding the effects of ALA on cachexia-related muscle atrophy, we performed a histological analysis of muscle fibre cross-sectional area. The gastrocnemius cross-sectional area was unaltered among the experimental groups, which could be explained by our experimental period of evaluation. Accordingly, Brown et al. showed a similar muscle fibre cross-sectional area frequency distribution 21 days after LLC inoculation, with significant differences only at 28 days of tumour implantation (222). Additionally, to better characterize LLC-associated muscle alterations, we analysed the myonuclei position, which is a sign of constant muscle fibre repair (223). LLC-hosts displayed an increased number of central myonuclei, a feature that has been previously observed in C26-bearing mice, as well as in pancreatic cancer patients (224,225). However, ALA failed to prevent the centrally positioned myonuclei. The absence of ALA effects on fibre diameter or myonuclei position might indicate an interference with other morphological parameters in the skeletal muscle, as an alternative explanation for the recovery of gastrocnemius wet weight.

Cancer-associated cachexia and type 2 diabetes (T2D) hold similar metabolic alterations (226), despite their obvious phenotypic differences. The activation of FFA1 and FFA4 receptors has been investigated as a pharmacological approach for the treatment of T2D, showing the relevance of both receptors in glycaemic control and in regulation of lipolytic pathways (227–229). Our data demonstrated that treatment with both ALA and the FFA1/FFA4 dual agonist GW9508 recovered the adipose tissue weight loss, whereas the also dual agonist TUG891 failed to recuperate the wet weight of adipose tissues. It is worth of mentioning that GW9508 has a 100-fold affinity for FFA1 than FFA4 (29), what might help to explain the different effects in comparison to TUG981. Interestingly, the selective antagonists of FFA1 and FFA4, GW1100 and AH7614, displayed mild effects on adipose tissue recovery in cachectic mice,

suggesting that both receptors are activated under cancer cachexia progression, contributing for the associated lipolysis. Of note, it is worth mentioning that adipose tissue lipolysis occurs before muscle atrophy in the LLC-cachexia model, and this could explain our distinct results regarding the lack of muscle fibres differences and the marked alterations in adipose tissue (230). Differences of FFA1 and FFA4 levels of activation might also help to explain whether both an agonist and two antagonists produced favourable effects on fat weight loss.

To further investigate adipose tissue modulation, we performed a histological analysis of adipocyte cross-sectional area of LLC-bearing mice treated with naturally-occurring or synthetic ligands that exerted a statistical effect on adipose tissue weight. The isWAT from mice is a mixed adipose tissue, characterized by the presence of white and brown adipose tissue (231). Nevertheless, we attempted to analyse the white adipose region from this fat depot with the intent to detect white adipocyte area alterations. The treatment with ALA, GW9508, and GW1100 altered the adipocyte cross-sectional area, corroborating the results obtained when this tissue weight was assessed. Also, the histological analysis of rWAT demonstrated that GW9508 treatment increased the number of adipocytes, supporting the increase of rWAT total weight, suggesting that the activation of FFA1 and FFA4 receptors is linked to adipocyte differentiation (213). Also, both antagonists, GW1100, and AH7614, increased the adipocyte area, upholding the obtained results from tissue weight assessment. Finally, we assessed the cross-sectional of epWAT from LLC-cachectic mice treated with GW9508, and it was possible to observe that this agonist also increased the number of smaller adipocytes, suggesting that FFA1/FFA4 activation might promote the adipocyte differentiation in white adipose tissue.

The UCP-1 is a mitochondrial protein associated with increased thermogenesis, and it has been associated with the pathophysiology of cancer-associated cachexia (147,153). To analyse the possible development of thermogenesis in fat depots of cachectic mice, we performed a Western blot assay to assess the UCP-1 expression in isWAT and epWAT. Interestingly, we were not able to detect UCP-1 in isWAT (results not shown). On the contrary, UCP-1 in epWAT was constitutively expressed in tumour-free control mice, whereas in LLC-bearing mice, this protein was upregulated, regardless of the GW9508 treatment. Supporting our results, a pancreatic cancer cachexia model displayed a time-dependent reduction of UCP-1 expression in brown adipose tissue of cachectic mice, accompanied by an overall decrease of this protein in white adipose tissue, when compared to tumour-free controls (232).

Cancer cachexia is associated with impaired quality of life, by reducing strength and exercise capacity, consequently, leading to a shorter survival (233,234). The development of cancer cachexia in our study led to a marked decrease in overall locomotor activity, corroborating previous assessments in LLC-bearing mice (235,236). We demonstrated that the FFA1/FFA4 dual agonist GW9508 improved the spontaneous locomotor activity in LLC cachectic mice in a significant manner, while DHA treatment slightly recovered this feature. Nonetheless, the other tested ligands did not alter this aspect. The FFA1 receptor activation has been associated with different behavioural alterations, such as stress-related pain and depression, as demonstrated by the treatment with DHA or GW9508 (41,179,237). Furthermore, the genic ablation of FFA1 receptors triggered distinct behavioural deficits, according to the evaluation of male and female mice (47,48). Additionally, the central activation of FFA4 receptors inhibited the food intake and reward, and chronically decreased anxiety-like behaviour in mice (64). It is worth of mentioning that FFA1 and FFA4 receptors are constitutively expressed in neurons and microglia, leading us to believe that the beneficial effects of GW9508 and DHA might rely on the activation of both receptors in the central nervous system (41,50,238).

We also evaluated the grip strength and motor coordination in LLC-bearing mice, as an additional measure of the decreased life quality in cachectic patients (234). We demonstrated that LLC-bearing mice displayed impaired motor coordination and grip strength. Similar data was formerly shown in mouse models of cancer cachexia induced by LLC or colorectal cancer inoculation (239,240). Regarding our results, we observed that DHA systemic treatment improved grip strength in LLC-bearing mice. In a mouse model of Duchenne muscular dystrophy, EPA/DHA supplementation restored grip strength, by modulating inflammation, oxidative stress, and sarcolemma stability (241). Additionally, DHA induced positive metabolic effects on skeletal muscle by FFA4 receptor activation (242). On the contrary, we also observed that LLC-bearing mice treated with the selective FFA4 antagonist AH7614 demonstrated improved grip strength, an effect that was not previously seen in any previous publications. This data reinforces the idea the different states of activation of FFA receptors can be observed in cachexia progression.

In virtue of the behavioural and metabolic impairments observed in LLC-cachectic mice in our study, we assessed, via microPET scanning analysis, the brain glucose metabolism by [18F]-FDG injection. Although we did not observe any change in whole brain glucose metabolism for any of the studied groups, LLC-hosts displayed a marked glucose hypometabolism in individually analysed brain regions, most

importantly in striatum, cortex, hypothalamus, superior colliculus and right inferior colliculus. In the MAC16 cachexia model, [18F]-FDG uptake was increased in brain and tumour of cachectic mice, contrasting with our results (196). Furthermore, in a cancer-anorexia rat model, cFos was expressed in the forebrain, which includes striatum and hypothalamus, indicating alterations in the catabolic pathways exerted by cachexia (243). Additionally, in non-small cell lung cancer patients, brain hypometabolism was observed in visual-spatial function-related areas; however, these patients were not cachectic (244). We further observed that GW9508 systemic treatment restored the cachexia-related hypometabolism. It is worth mentioning that FFA1 is highly expressed in the hypothalamus, hippocampus, medulla oblongata, cerebellum, olfactory bulb, striatum, cerebral cortex, spinal cord, and astrocytes while the FFA4 receptor expression was observed in the whole brain, more specifically, in the hypothalamus and pituitary gland (42,44,245–247). Moreover, in a mouse model of diabesity, the chronic central administration of GW9508 improved the cognitive performance by activating hippocampal FFA1 receptors (52). Also, the chronic activation of brain FFA4 by centrally dosing the selective agonist GPR120III improved anxiety-like behaviour in mice (64). We further analysed [18F]-FDG biodistribution in tumour and brain from LLC-bearing mice and, regardless of the GW9508 treatment, [18F]-FDG uptake was unaltered, demonstrating that brain glucose hypometabolism was indeed due to the cachectic process, rather than to tumour growth. We also evaluated the effects of ALA and DHA in cachexia-related hypometabolism, but they failed to rescue this impairment. This might be related to the metabolism of both naturally-occurring ligands, as the secondary products might not be generated in sufficient levels to modulate brain hypometabolism.

The FFA1 and FFA4 receptors are constitutively expressed in different tissues that are mainly involved in metabolism regulation (248). The FFA1 receptor expression was previously detected in pancreas, brain, muscle, spinal cord, osteoblasts, osteoclasts, macrophages, and fibroblasts (41,245,249–251). As for FFA4 receptor, its expression was detected in adipose tissue, gastrointestinal tract, muscle, liver, retina, brain, aorta, and taste buds (58,65,182–184,238,249). It is worth mentioning that the expression of both receptors was investigated by different techniques, regularly showing their expression by mRNA or immunofluorescence. Herein, we demonstrated the immunopositivity of FFA1 receptors in adipose tissue by immunohistochemistry. In isWAT, we showed that FFA1 receptor is upregulated in the presence of cachexia, suggesting its participation in cachexia development. Similarly, in a rat model of spinal nerve ligation, FFA1 receptor was upregulated in microglia, astrocytes and neurons of the spinal cord (42). Conversely, the FFA1 receptor was downregulated in the spinal cord in a mouse model of visceral

pain, whereas the systemic administration of DHA restored this parameter (41). As for the FFA4 immunopositivity, we observed that this receptor is expressed in isWAT and epWAT of mice, regardless of LLC-cachexia induction. However, Quesada-Lopez et al (65) demonstrated that FFA4 mRNA expression in adipose tissue was upregulated by cold exposure. Also, it was previously shown that mice with genetic ablation of FFA4 develop obesity, glucose intolerance, decreased adipocyte differentiation and lipogenesis. Furthermore, the same study showed that FFA4 gene expression is upregulated in obese humans, when compared to lean individuals (61). Based on our findings, one might suggest that FFA1 participates, in a higher extent, of cancer cachexia development more than FFA4; however, both receptors are likely essential for the development and treatment of this condition. It was demonstrated that dietary omega-3 activates FFA4 receptor in adipose tissues of lean mice, indicating the effectiveness of omega-3 fatty acids to modulate this receptor (249). The same study demonstrated that gastrocnemius FFA1receptor is also activated by dietary omega-3. We demonstrated by Western blotting, that FFA1 protein expression is upregulated in the gastrocnemius of LLC-cachectic mice, and this was reversed by the FFA1/FFA4 dual agonist GW9508 systemic treatment. Of note, the FFA1 upregulation in both adipose and muscle tissues might indicate a muscle-adipose tissue crosstalk, since this is considered a hallmark of cancer cachexia (171). In our study, the FFA1 receptor was found constitutively expressed in pancreas, regardless of cachexia development. Contrariwise, it was previously demonstrated that FFA1 receptor expression is diminished in the pancreas of diabetic mice (229,252). Thus, we can suggest that FFA1 expression does not depend on cancer cachexia development. It is important to mention that we investigated the adipose tissue expression of FFA4 only by immunohistochemistry, and not by Western blotting, in virtue of the low blot quality and non-specific protein expression.

Our findings demonstrate, for the first time, the relevance of both FFA1and FFA4 receptors in cancer-associated cachexia. These receptors partly mediate the beneficial effects induced by the parenteral administration of ALA and DHA, despite additional sites of action for both naturally-occurring compounds cannot be discarded. Herein, we observed overlapping effects for synthetic FFA1/FFA4 agonists and antagonists in the mouse model of LLC-induced cachexia. A previous study pointed out opposing beneficial or detrimental actions for FFA1 receptors in several pathophysiological responses, supporting somewhat our data (112). Indeed, it has been suggested that FFA4 receptor co-activation potentiates FFA1 receptor-mediated antidiabetic effects in rodents, further indicating a redundancy of actions mediated by the two receptors (80). Noteworthy, the dual FFA1/FFA4 agonist GW9508, which displays a preference for FFA1

receptor, exhibited superior responses regarding the control of peripheral and central alterations secondary to cachexia induction. Accordingly, we showed an upregulation of FFA1 receptors in adipose tissue and skeletal muscle of LLC-hosts, upholding a most relevant role for FFA1 receptors, in our experimental paradigm. Nevertheless, targeting both FFA1and FFA4 receptors appears to represent an attractive strategy for cachexia management.

Acknowledgements

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Table 1 Effects of ALA and DHA on cancer-associated cachexia body alterations.

	Tumour free-control group				LLC-group			
	Control	**ALA**		**DHA**	**Control**	**ALA**		**DHA**
	-	**2.5 mg/kg**	**5 mg/kg**	**1 µmol/kg**		**2.5 mg/kg**	**5 mg/kg**	**1 µmol/kg**
Gastrocnemius (mg)	139±3	134±3	136±6	133±7	117±4[a]	136±4[b]	140±2[b]	123±7
Tibialis anterior (mg)	58±3	61±5	54±2	51±2	44±3[a]	53±3	53±2	41±2[a]
Soleus (mg)	8±1	8±1	6±1	7±1	7±1	7±1	7±1	8±1
isWAT (mg)	71±6	83±9	67±7	71±3	40±4[a]	77±12[b]	63±6	62±10
rWAT (mg)	55±7	76±9	51±5	48±4	36±4	50±8	36±4	35±7
epWAT (mg)	176±12	125±20	191±46	132±21	108±11[a]	109±11	115±6	166±13

isWAT, intrascapular white adipose tissue; ingWAT, inguinal white adipose tissue; rWAT, retroperitoneal white adipose tissue; epWAT, epididymal white adipose tissue; ALA, α-linolenic acid; DHA, docosahexaenoic acid. Data represents the mean±SEM of 8-24 animals/group.

[a]Denotes statistical difference from tumour free-control group

[b]Denotes statistical difference from LLC-control group

Table 2 Effects of the FFA1/FFA4 dual agonist GW9508 and the FFA1 selective antagonist GW1100 on cancer-associated cachexia body alterations.

	Tumour free-control group					**LLC-group**				
	Control	**GW9508**		**GW1100**		**Control**	**GW9508**		**GW1100**	
	-	**2 mg/kg**	**8 mg/kg**	**4 mg/kg**	**10 mg/kg**		**2 mg/kg**	**8 mg/kg**	**4 mg/kg**	**10 mg/kg**
Gastrocnemius (mg)	147±3	130±5	144±3	147±4	149±5	137±4	118±6[ab]	137±4	141±5	144±8
Tibialis anterior (mg)	60±4	54±3	57±3	59±6	76±4	53±3	43±4[a]	58±4	57±6	57±4
Soleus (mg)	8±1	8±1	6±1	7±1	8±1	8±1	7±1	8±1	8±1	8±1
isWAT (mg)	56±5	55±5	99±10	114±14	75±6	51±5	42±5	81±7[ab]	84±7[ab]	56±7
rWAT (mg)	47±3	35±3	48±4	62±4	41±4	27±3[a]	21±4[a]	41±5[b]	43±4[b]	20±3[a]
epWAT (mg)	200±14	197±2	199±14	255±20	188±27	116±11[a]	110±18[a]	200±23[b]	149±12	141±23

isWAT, intrascapular white adipose tissue; ingWAT, inguinal white adipose tissue; rWAT, retroperitoneal white adipose tissue; epWAT, epididimal white adipose tissue; Data represents the mean±SEM of 8-24 animals/group.

[a]Denotes statistical difference from tumor free-control group

[b]Denotes statistical difference from LLC-control group

Table 3 Effects of the FFA1/FFA4 dual agonist TUG891 and FFA4 selective antagonist AH7614 on cancer-associated cachexia body alterations.

	Tumour free-control group					**LLC-group**				
	Control	**TUG891**		**AH7614**		**Control**	**TUG891**		**AH7614**	
	-	**2 mg/kg**	**8 mg/kg**	**4 mg/kg**	**10 mg/kg**		**2 mg/kg**	**8 mg/kg**	**4 mg/kg**	**10 mg/kg**
Gastrocnemius (mg)	150±5	144±8	152±4	133±5	160±6	135±4	127±5[a]	139±4	139±4	119±4[a]
Tibialis anterior (mg)	71±3	68±3	71±1	55±3	75±4	59±4	62±3	68±3	54±3	54±3
Soleus (mg)	7±1	8±1	6±1	7±1	7±1	7±1	6±1	7±1	8±1	6±1
isWAT (mg)	72±6	79±7	67±5	56±3	77±5	42±3[a]	56±5	55±4	45±5[a]	42±6[a]
rWAT (mg)	42±4	45±7	44±5	47±7	41±4	24±4[a]	16±3[a]	29±4	45±6[b]	18±3[a]
epWAT (mg)	211±6	261±32	220±13	204±32	248±13	133±15[a]	153±12	189±16	193±27	169±16

isWAT, intrascapular white adipose tissue; ingWAT, inguinal white adipose tissue; rWAT, retroperitoneal white adipose tissue; epWAT, epididymal white adipose tissue; Data represents the mean±SEM of 8-24 animals/group.

[a]Denotes statistical difference from tumour free-control group

[b]Denotes statistical difference from LLC-control group

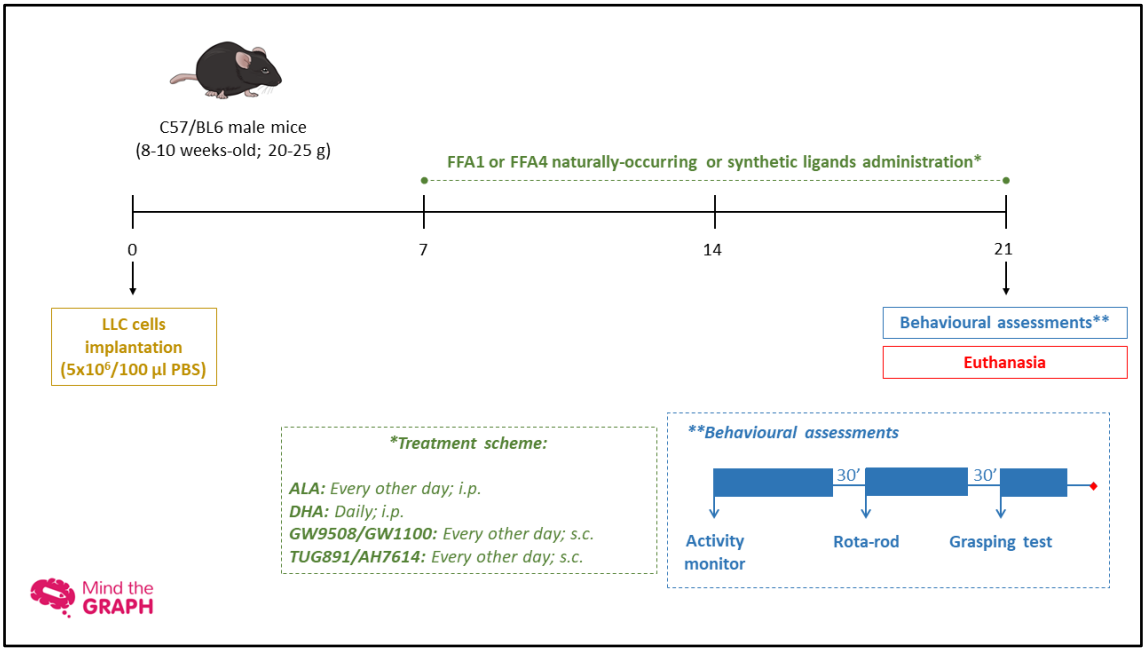


Fig 1 Schematic figure of the tumour induction and treatment protocols. Lewis lung carcinoma (LLC)-cells were injected into the right hind flank of C57/BL6 mice. Seven days after tumour implantation, mice were submitted to the respective treatment schemes, until the 21st day. On this last day, behavioural experiments were performed, and mice were euthanized for sample collection. PBS, phosphate-buffered saline.

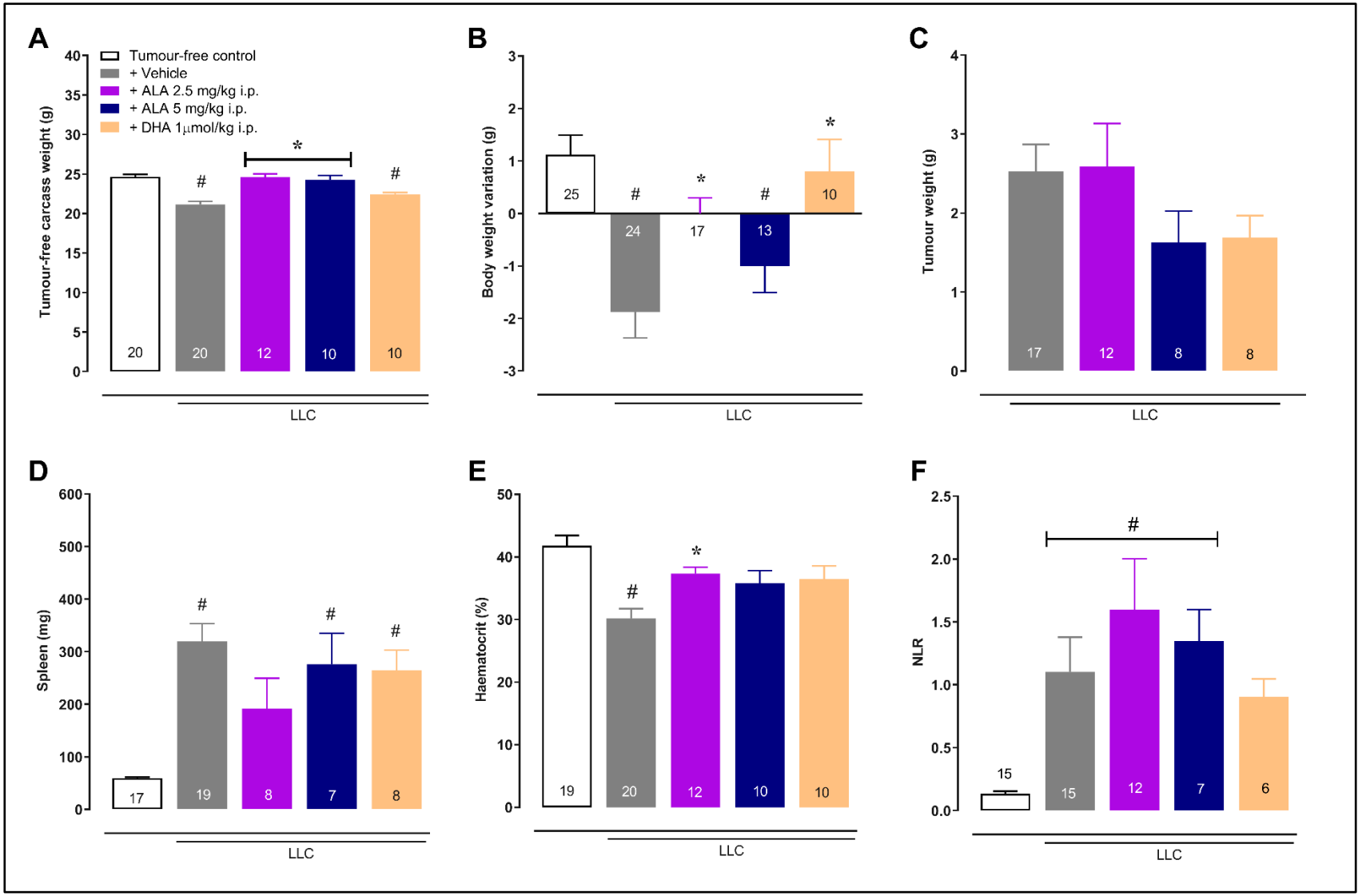

A
Tumour-free control
+ Vehicle
+ ALA 2.5 mg/kg i.p.
+ ALA 5 mg/kg i.p.
+ DHA 1μmol/kg i.p.
Tumour-free carcass weight (g)
40
35
30
25
20
15
10
5
0
20
20
12
10
10
#
*
#
LLC
B
Body weight variation (g)
3
2
1
0
-1
-2
-3
25
24
17
13
10
#
*
#
*
LLC
C
Tumour weight (g)
4
3
2
1
0
17
12
8
8
LLC
D
Spleen (mg)
600
500
400
300
200
100
0
17
19
8
7
8
#
#
#
LLC
E
Haematocrit (%)
50
40
30
20
10
0
19
20
12
10
10
#
*
LLC
F
NLR
2.5
2.0
1.5
1.0
0.5
0.0
15
15
12
7
6
#
LLC

Fig. 2 Phenotypic and inflammatory outcomes in LLC-cachexia model, after omega-3 fatty acids i.p. treatment. (A) Tumour-free carcass weight, (B) body weight variation throughout 21 days, and (C) tumour weight was assessed after ALA or DHA treatment. (D) Spleen weight was measured, and (E) haematocrit and (F) neutrophil-lymphocyte-ratio (NLR) were evaluated afterwards, using total blood. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

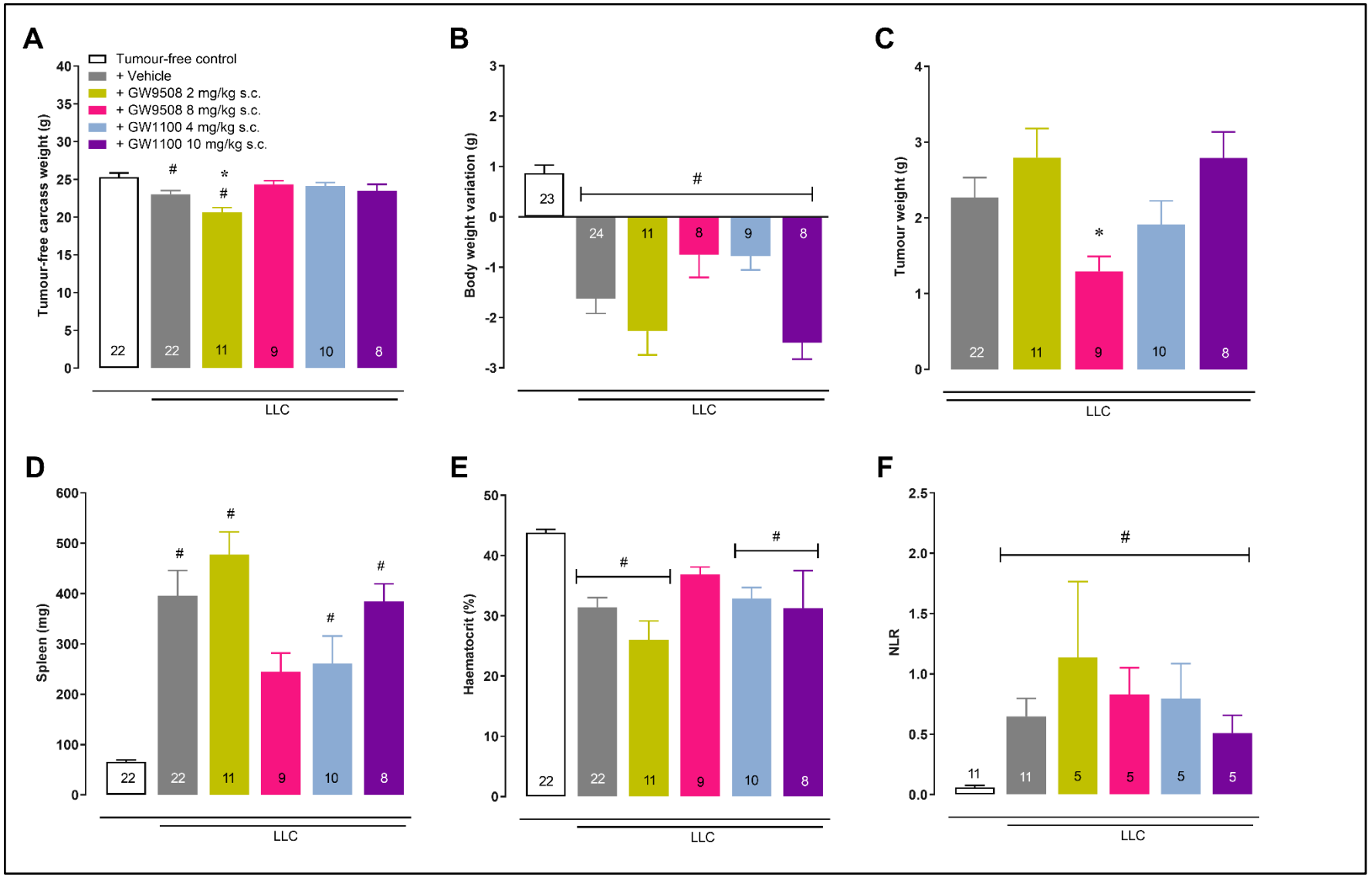

A
Tumour-free control
+ Vehicle
+ GW9508 2 mg/kg s.c.
+ GW9508 8 mg/kg s.c.
+ GW1100 4 mg/kg s.c.
+ GW1100 10 mg/kg s.c.
Tumour-free carcass weight (g)
40
35
30
25
20
15
10
5
0
#
*
#
22
22
11
9
10
8
LLC
B
Body weight variation (g)
3
2
1
0
-1
-2
-3
#
23
24
11
8
9
8
LLC
C
Tumour weight (g)
4
3
2
1
0
*
22
11
9
10
8
LLC
D
Spleen (mg)
600
500
400
300
200
100
0
#
#
#
#
22
22
11
9
10
8
LLC
E
Haematocrit (%)
50
40
30
20
10
0
#
#
22
22
11
9
10
8
LLC
F
NLR
2.5
2.0
1.5
1.0
0.5
0.0
#
11
11
5
5
5
5
LLC

Fig. 3 Phenotypic and inflammatory characteristics of LLC-injected mice, after the administration of the partial agonist or the selective antagonist of FFA1, GW9508 (2 or 8 mg/kg, s.c.) or GW1100 (4 or 10 mg/kg, s.c.), respectively. (A) Tumour-free carcass weight, (B) body weight variation throughout 21 days, and (C) tumour weight were assessed after GW9508 or GW1100 treatment. (D) Spleen weight was measured, and (E) haematocrit and (F) neutrophil-lymphocyte-ratio (NLR) were evaluated afterwards, using total blood. Parameters are expressed as mean ± SEM. #$P<0.05$ denotes difference from control group; *$P<0.05$ denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

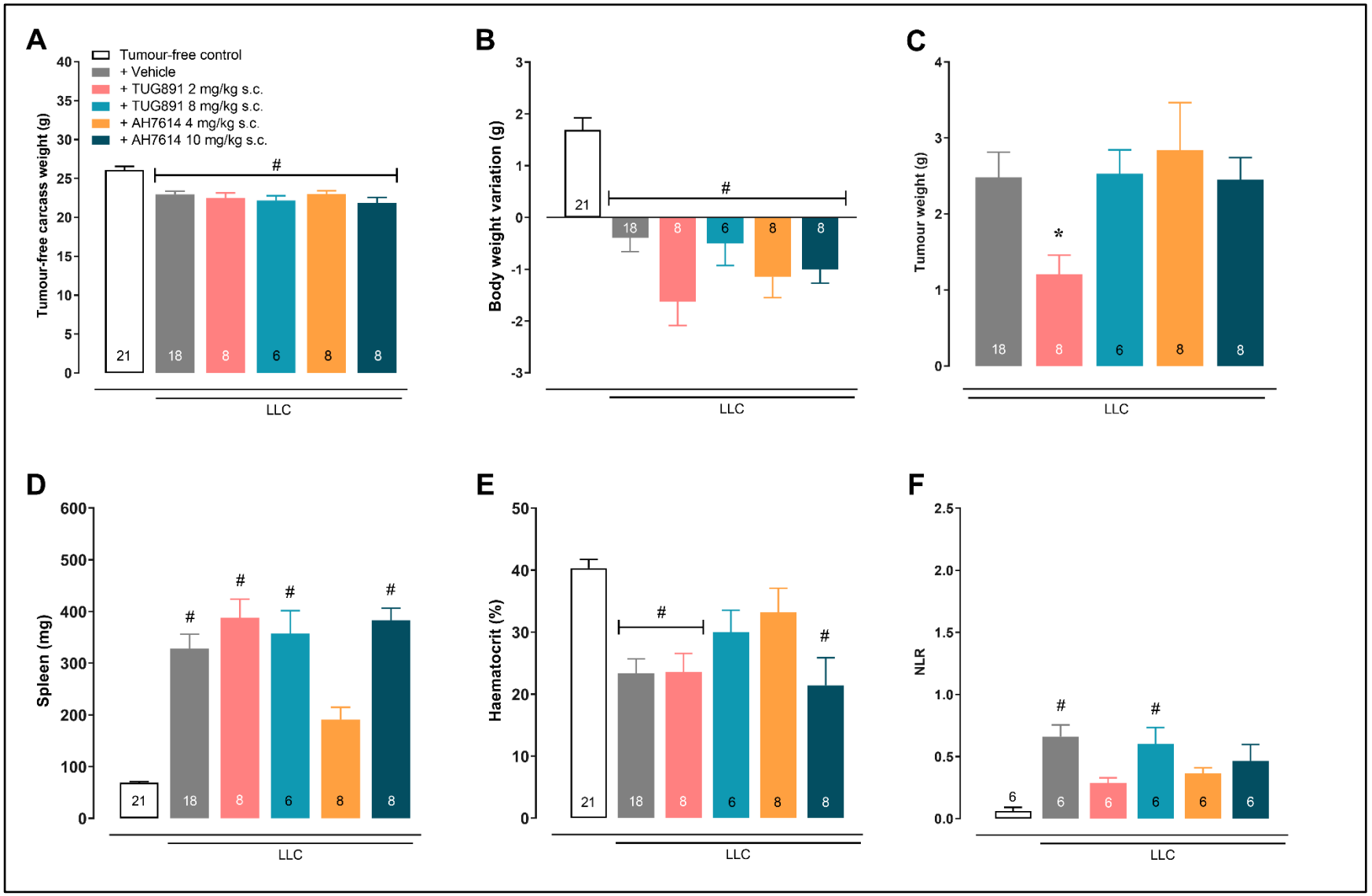

A
Tumour-free control
+ Vehicle
+ TUG891 2 mg/kg s.c.
+ TUG891 8 mg/kg s.c.
+ AH7614 4 mg/kg s.c.
+ AH7614 10 mg/kg s.c.
Tumour-free carcass weight (g)
LLC
B
Body weight variation (g)
LLC
C
Tumour weight (g)
LLC
D
Spleen (mg)
LLC
E
Haematocrit (%)
LLC
F
NLR
LLC

Fig. 4 Phenotypic and inflammatory aspects of LLC-injected mice, after the administration of the partial agonist or the selective antagonist of FFA4, TUG891 (2 or 8 mg/kg, s.c.) or AH7614 (4 or 10 mg/kg, s.c.), respectively. (A) Tumour-free carcass weight, (B) body weight variation throughout 21 days, and (C) tumour weight were assessed after TUG891 or AH7614 treatment. (D) Spleen weight was measured, and (E) haematocrit and (F) neutrophil-lymphocyte-ratio (NLR) were evaluated afterwards, using total blood. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

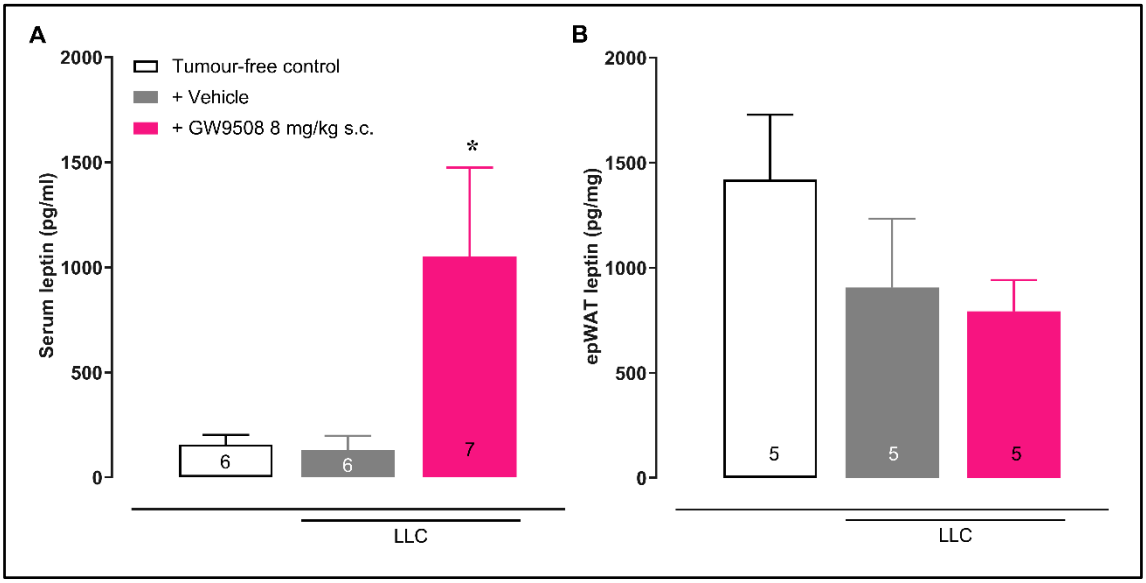


Fig. 5 Effects of the treatment with GW9508 in LLC-bearing mice on circulating and tissue leptin levels. The levels of (A) serum and (B) epWAT leptin were assessed in LLC-bearing mice that received repeated treatment with GW9508 (8 mg/kg, s.c.). Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

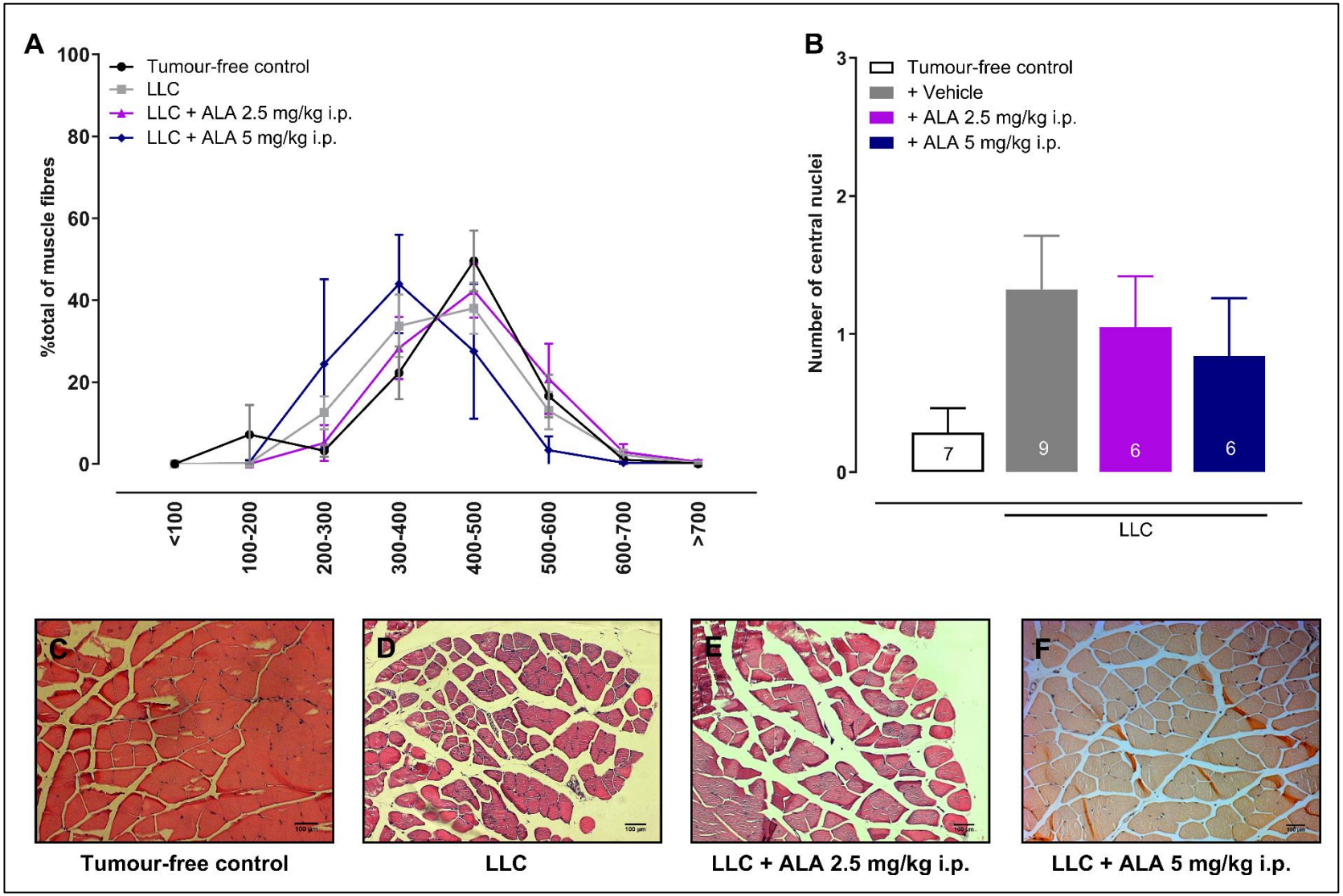

A
100
80
60
40
20
0
%total of muscle fibres
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg i.p.
LLC + ALA 5 mg/kg i.p.
<100
100-200
200-300
300-400
400-500
500-600
600-700
>700
B
3
2
1
0
Number of central nuclei
Tumour-free control
+ Vehicle
+ ALA 2.5 mg/kg i.p.
+ ALA 5 mg/kg i.p.
7
9
6
6
LLC
C
D
E
F
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg i.p.
LLC + ALA 5 mg/kg i.p.

Fig. 6 Skeletal muscle alterations associated to cancer-cachexia, after systemic ALA treatment. (A) The fibre size frequency distribution and (B) the number of central myonuclei of gastrocnemius muscle were evaluated after 21 days of tumour growth. Representative images of the gastrocnemius muscle for (C) tumour-free control mice, (D) LLC-bearing mice and LLC-treated mice with (E) ALA 2.5 mg/kg or (F) 5 mg/kg, given i.p (200x magnification). Parameters are expressed as mean ± SEM.

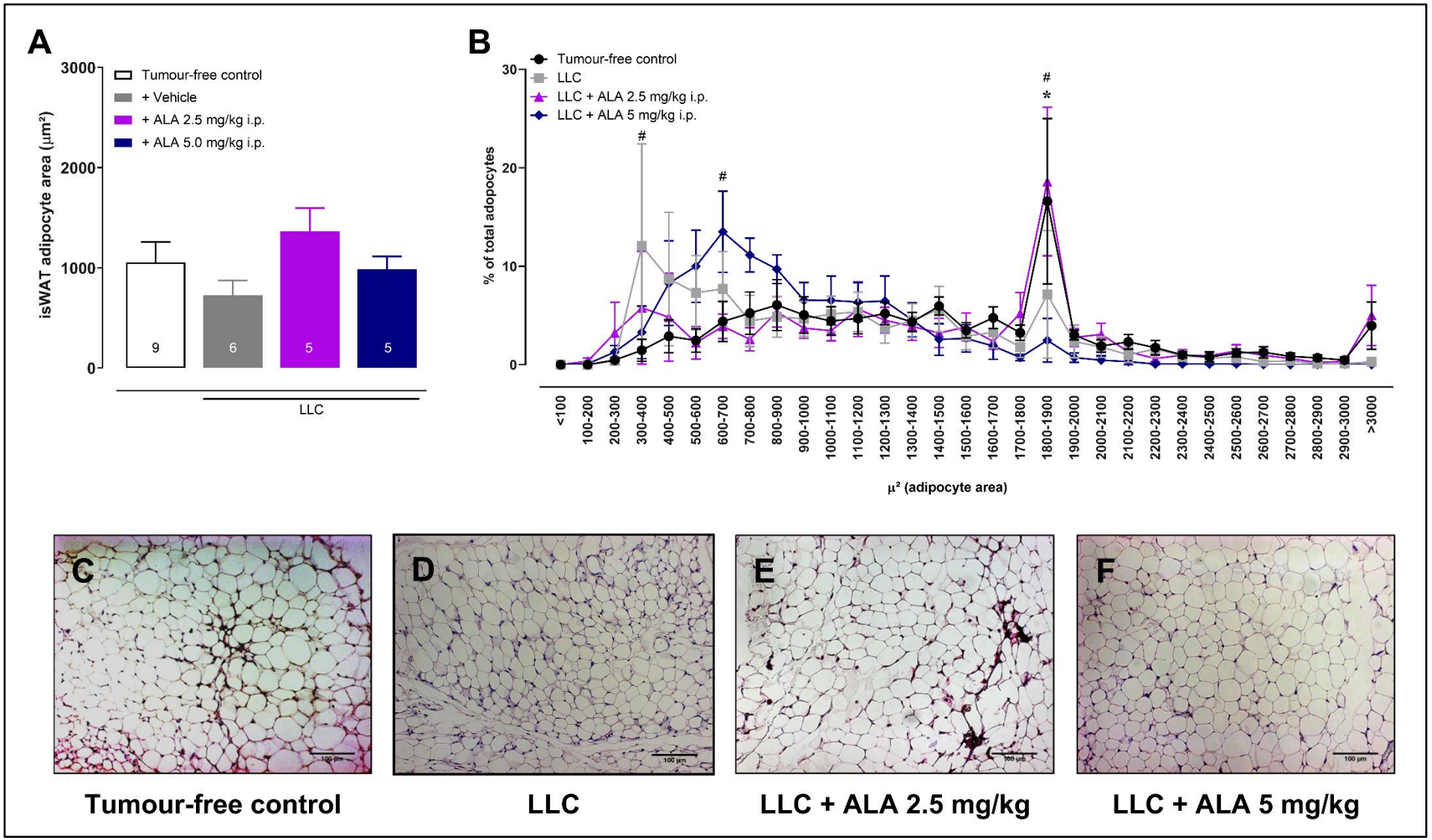

A
3000
2000
1000
0
isWAT adipocyte area (μm²)
Tumour-free control
+ Vehicle
+ ALA 2.5 mg/kg i.p.
+ ALA 5.0 mg/kg i.p.
9
6
5
5
LLC
B
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg i.p.
LLC + ALA 5 mg/kg i.p.
30
20
10
0
% of total adopocytes
#
#
#
*
<100
100-200
200-300
300-400
400-500
500-600
600-700
700-800
800-900
900-1000
1000-1100
1100-1200
1200-1300
1300-1400
1400-1500
1500-1600
1600-1700
1700-1800
1800-1900
1900-2000
2000-2100
2100-2200
2200-2300
2300-2400
2400-2500
2500-2600
2600-2700
2700-2800
2800-2900
2900-3000
>3000
μ² (adipocyte area)
C
D
E
F
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg
LLC + ALA 5 mg/kg

Fig. 7 Adipocyte cross-sectional area from isWAT of ALA-treated LLC-bearing mice. (A) Average adipocyte cross-sectional area and (B) adipocyte area relative frequency from LLC-injected mice treated with ALA (2.5 mg/kg or 5 mg/kg, i.p.). Representative images of isWAT from (C) tumour-free control mice, (D) LLC-bearing mice, LLC-treated mice with (E) ALA 2.5 mg/kg i.p., and (F) ALA 5 mg/kg, i.p (200x magnification). Parameters are shown as mean ± SEM. #P<0.05 denotes significance from tumour-free control group; *P<0.05 denotes significance from LLC-bearing group (Two-way ANOVA followed by Tukey's post hoc test).

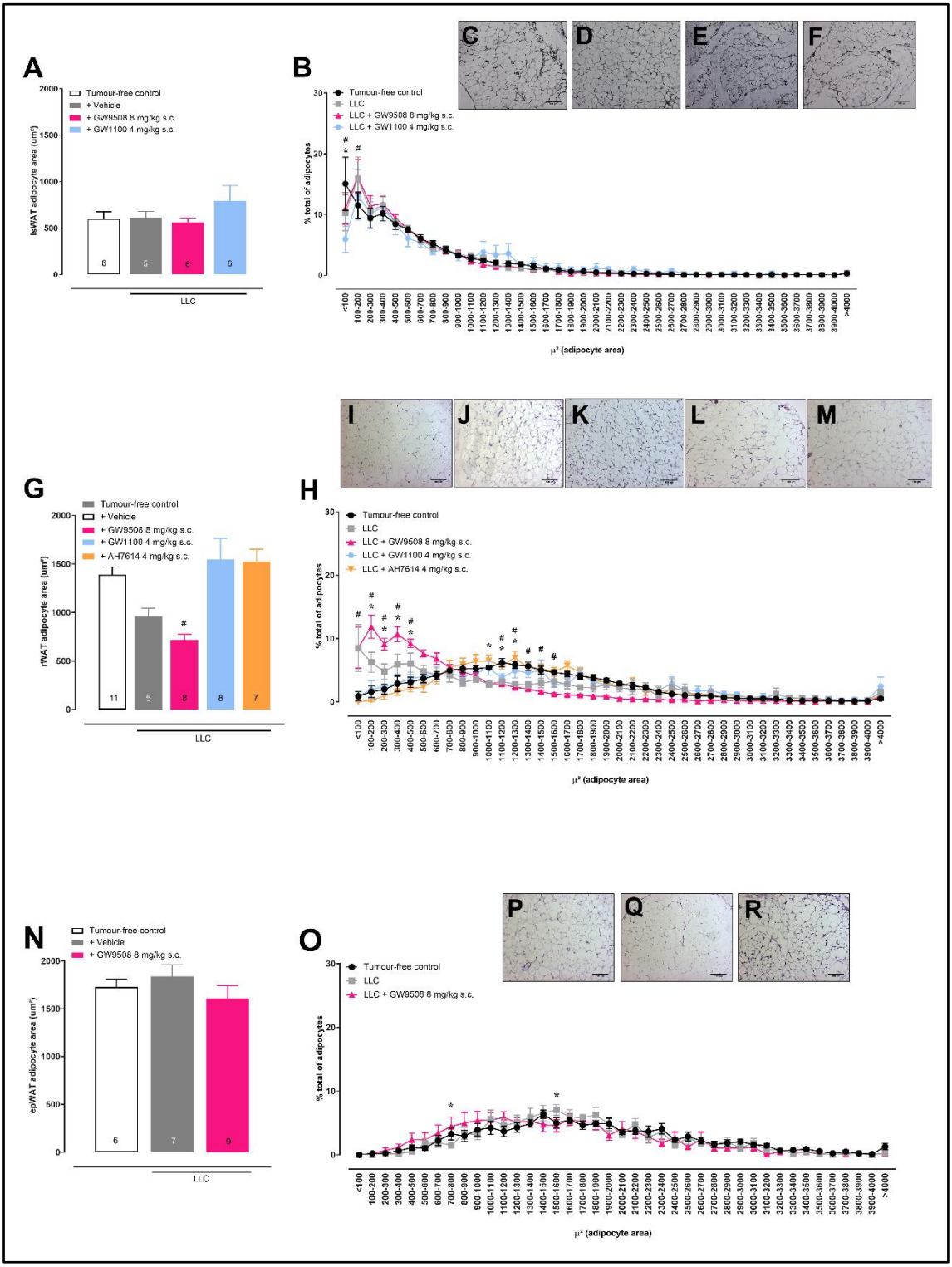


Fig. 8 Effects of the FFA1 and FFA4 synthetic ligands in the adipocyte cross-sectional area in LLC-bearing mice. (A) Average adipocyte cross-sectional area and (B) adipocyte area frequency distribution from LLC-injected mice treated with GW9508 (8 mg/kg, s.c.)

or GW1100 (4 mg/kg, s.c.). Representative images of rWAT from (C) tumour-free control mice, (D) LLC-bearing mice, LLC-treated mice with (E) GW9508 (8 mg/kg, s.c.), and (F) GW1100 (4 mg/kg, s.c.) (200x magnification). (G) Average adipocyte cross-sectional area and (H) adipocyte cross-sectional frequency distribution from LLC-mice treated with GW9508 (8 mg/kg, s.c.), GW1100 (4 mg/kg, s.c.) or AH7614 (4 mg/kg, s.c.) Representative images of rWAT from (I) tumour-free control mice, (J) LLC-bearing mice and LLC-treated mice with (K) GW9508 (8 mg/kg, s.c.), (L) GW1100 (4 mg/kg, s.c.) and (M) AH7614 (4 mg/kg, s.c.). (N) Average adipocyte cross-sectional area and (O) adipocyte area frequency distribution from LLC-bearing mice treated with GW9508 (8 mg/kg, s.c.). Representative images of (P) tumour-free control mice, (Q) LLC-bearing mice and (R) LLC-treated with GW9508. Parameters are shown as mean ± SEM. #$P<0.05$ denotes significance from tumour-free control group; *$P<0.05$ denotes significance from LLC-bearing group (Two-way ANOVA followed by Tukey's post hoc test).

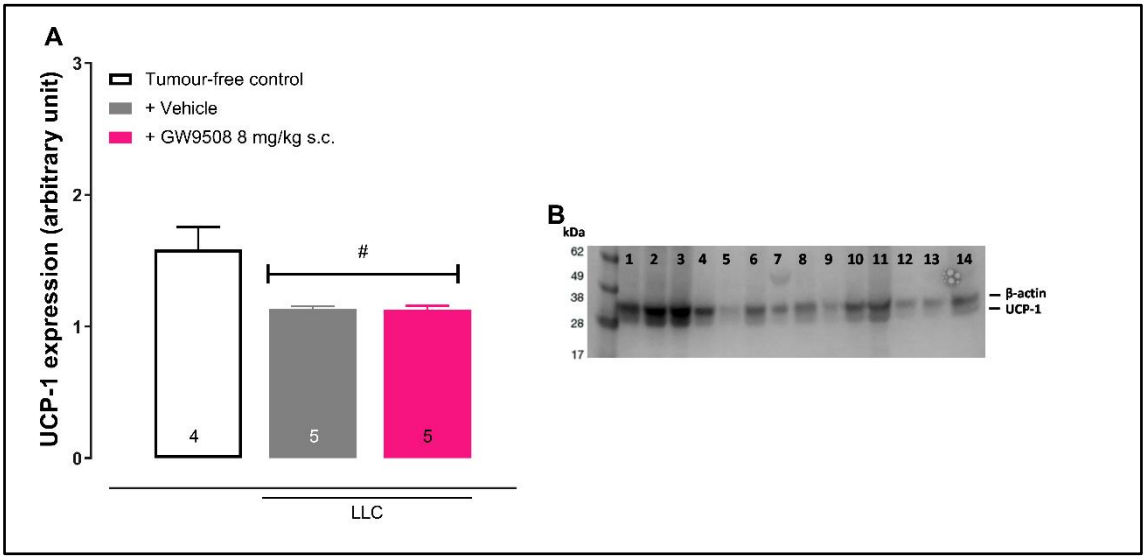


Fig. 9 Effects of the repeated treatment with GW9508 (8 mg/kg, s.c.) on UCP-1 protein expression and their representative blots in visceral adipose tissue from LLC-bearing mice. (A) Analysis of UCP-1 expression in epWAT of LLC-injected mice, with or without treatment with GW9508 (8 mg/kg, s.c.), and the respective tumour-free control. (B) Representative blots of UCP-1 protein expression in epWAT from tumour-free control mice (lanes 1-4), LLC-bearing mice (lanes 5-9) and LLC-bearing mice treated with GW9508 (lanes 10-14). Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group (One-way ANOVA followed by Bonferroni's post hoc test).

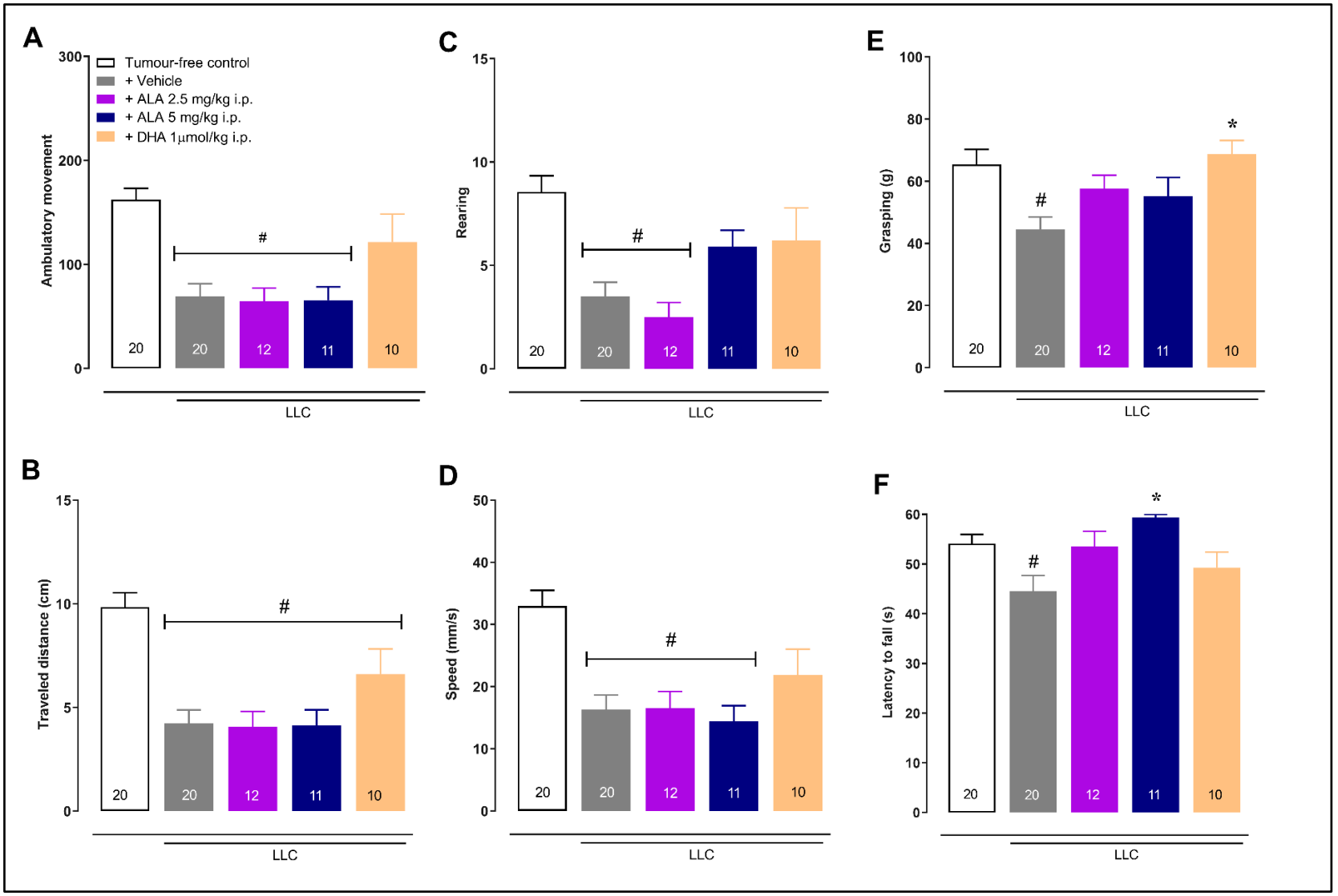

A
Tumour-free control
+ Vehicle
+ ALA 2.5 mg/kg i.p.
+ ALA 5 mg/kg i.p.
+ DHA 1μmol/kg i.p.
Ambulatory movement
300
200
100
0
#
20
20
12
11
10
LLC
C
Rearing
15
10
5
0
#
20
20
12
11
10
LLC
E
Grasping (g)
100
80
60
40
20
0
#
*
20
20
12
11
10
LLC
B
Traveled distance (cm)
15
10
5
0
#
20
20
12
11
10
LLC
D
Speed (mm/s)
50
40
30
20
10
0
#
20
20
12
11
10
LLC
F
Latency to fall (s)
60
50
40
30
20
10
0
#
*
20
20
12
11
10
LLC

Fig. 10 Characterization of spontaneous locomotor activity, strength and motor coordination in the mouse model of LLC-induced cachexia, after omega-3 fatty acids parenteral administration. Spontaneous locomotor activity was evaluated 21 days after tumor implantation and characterized by (A) ambulatory movement, (B), travelled distance, (C) rearing and (D) speed, after the repeated systemic treatment with ALA (2.5 or 5 mg/kg, i.p.) or DHA (1 μmol/kg, i.p.). (E) Strength was evaluated by grasping and (F) motor coordination was assessed by rota-rod test, after 21 days of tumor cell injection and i.p administration of ALA or DHA. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

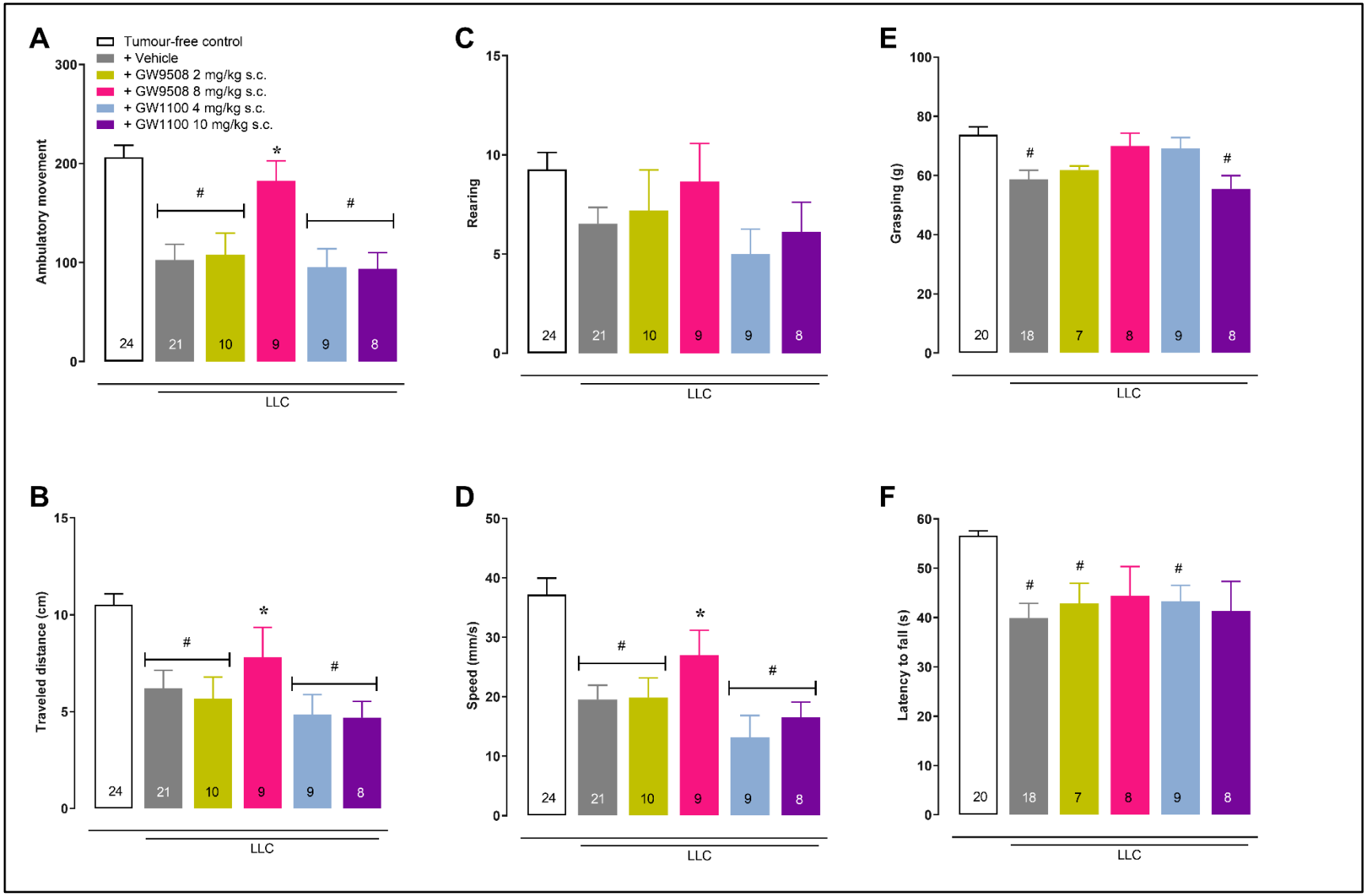

A
Tumour-free control
+ Vehicle
+ GW9508 2 mg/kg s.c.
+ GW9508 8 mg/kg s.c.
+ GW1100 4 mg/kg s.c.
+ GW1100 10 mg/kg s.c.
Ambulatory movement
300
200
100
0
24
21
10
9
9
8
#
*
#
LLC
C
Rearing
15
10
5
0
24
21
10
9
9
8
LLC
E
Grasping (g)
100
80
60
40
20
0
20
18
7
8
9
8
#
#
LLC
B
Traveled distance (cm)
15
10
5
0
24
21
10
9
9
8
#
*
#
LLC
D
Speed (mm/s)
50
40
30
20
10
0
24
21
10
9
9
8
#
*
#
LLC
F
Latency to fall (s)
60
50
40
30
20
10
0
20
18
7
8
9
8
#
#
#
LLC

Fig. 11 Assessment of spontaneous locomotor activity, grip strength and motor coordination of LLC-injected mice, after administration of GW9508 (2 or 8 mg/kg, s.c.) or GW1100 (4 or 10 mg/kg, s.c.). (A) Ambulatory movement, (B) travelled distance, (C) rearing and (D) speed were appraised after the repeated s.c. treatment with GW9508 or GW1100. (E) Grip strength and (F) motor coordination were evaluated after the repeated s.c. injection of GW9508 or GW1100. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

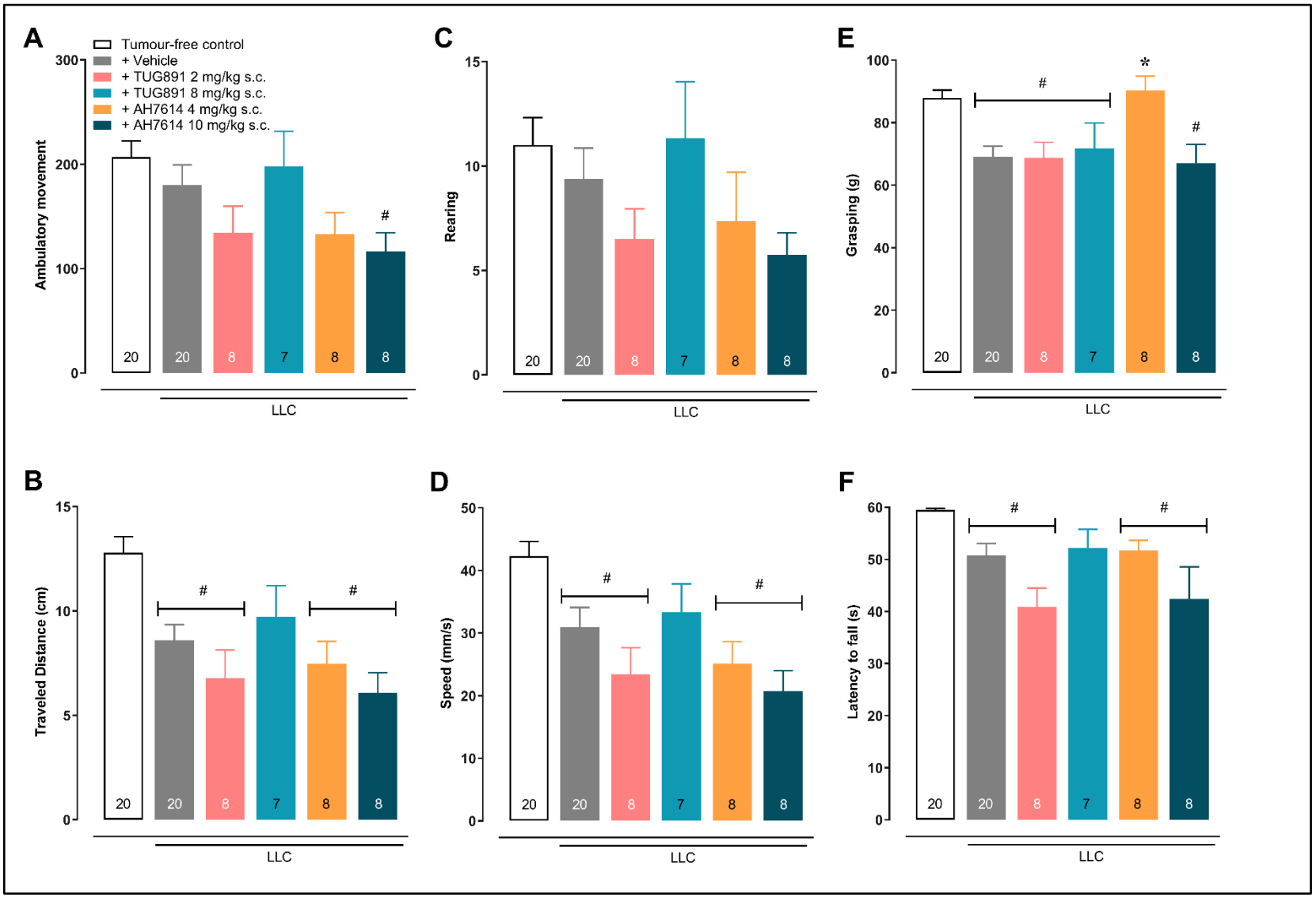

A
Tumour-free control
+ Vehicle
+ TUG891 2 mg/kg s.c.
+ TUG891 8 mg/kg s.c.
+ AH7614 4 mg/kg s.c.
+ AH7614 10 mg/kg s.c.
Ambulatory movement
300
200
100
0
20
20
8
7
8
8
#
LLC
C
Rearing
15
10
5
0
20
20
8
7
8
8
LLC
E
Grasping (g)
100
80
60
40
20
0
#
*
#
20
20
8
7
8
8
LLC
B
Traveled Distance (cm)
15
10
5
0
#
#
20
20
8
7
8
8
LLC
D
Speed (mm/s)
50
40
30
20
10
0
#
#
20
20
8
7
8
8
LLC
F
Latency to fall (s)
60
50
40
30
20
10
0
#
#
20
20
8
7
8
8
LLC

Fig. 12 Evaluation of spontaneous locomotor activity, grip strength and motor coordination of LLC-injected mice, after administration of TUG891 (2 or 8 mg/kg, s.c.) or AH7614 (4 or 10 mg/kg, s.c.). (A) Ambulatory movement, (B) travelled distance, (C) rearing and (D) speed were appraised after the repeated s.c. treatment with TUG891 or AH7614. (E) Grip strength and (F) motor coordination were evaluated after the repeated s.c. injection of TUG891 or AH7614. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group; *P<0.05 denotes difference from LLC + Vehicle group (One-way ANOVA followed by Bonferroni's post hoc test).

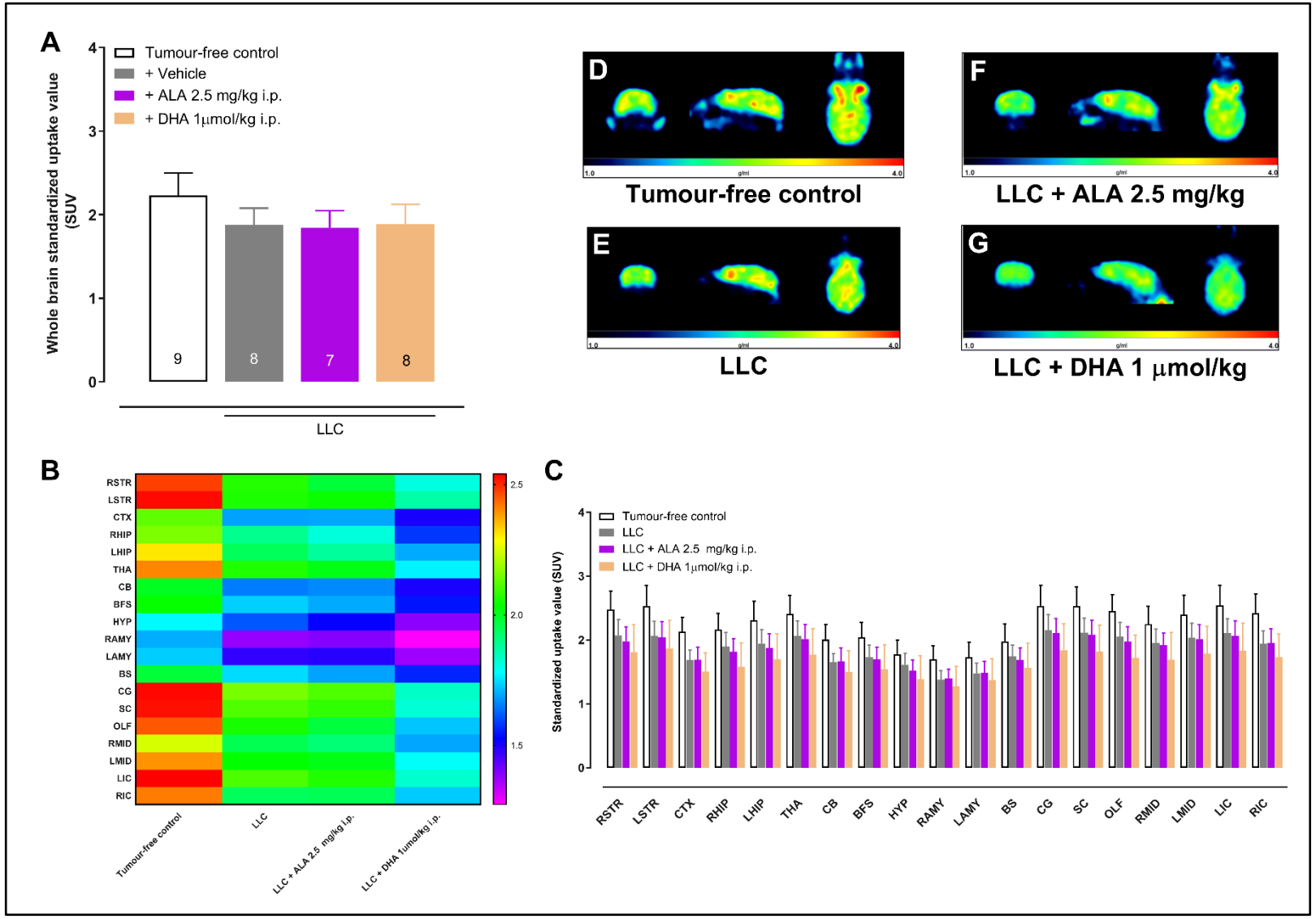

A
Tumour-free control
+ Vehicle
+ ALA 2.5 mg/kg i.p.
+ DHA 1μmol/kg i.p.
Whole brain standardized uptake value (SUV)
9
8
7
8
LLC
D
Tumour-free control
F
LLC + ALA 2.5 mg/kg
E
LLC
G
LLC + DHA 1 μmol/kg
B
RSTR
LSTR
CTX
RHIP
LHIP
THA
CB
BFS
HYP
RAMY
LAMY
BS
CG
SC
OLF
RMID
LMID
LIC
RIC
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg i.p.
LLC + DHA 1umol/kg i.p.
C
Tumour-free control
LLC
LLC + ALA 2.5 mg/kg i.p.
LLC + DHA 1μmol/kg i.p.
Standardized uptake value (SUV)

Fig. 13 Effects of the repeated treatment with ALA (2.5 mg/kg, i.p.) or DHA (1 μmol/kg, i.p.) on the [18F]-FDG brain hypometabolism associated to cancer-induced cachexia. (A) Whole brain metabolism evaluation after 21 days of tumour implantation. The panels B and C show the glucose uptake throughout different brain regions, as a heat map or in columns, respectively. Parameters are expressed as mean ± SEM. Representative images of (D) tumour-free control mice, (E) LLC-bearing mice, (F) cancer cachectic mice treated with ALA (2.5 mg/kg, i.p.) and (G) cancer cachectic mice treated with DHA (1 umol/kg, i.p.). Abbreviations: RSTR, right striatum region; LSTR, left striatum region; CTX, cortex; RHIP, right hippocampal region; LHIP, left hippocampal region; THA, thalamus; CB, cerebellum; BFS, basal forebrain/septum; HYP, hypothalamus; RAMY, right amygdala; LAMY, left amygdala; BS, brainstem; CG, cingulate gyrus; SC, superior colliculus; OLF, olfactory areas; RMID, right midbrain; LMID, left midbrain; LIC, left inferior colliculus; RIC, right inferior colliculus.

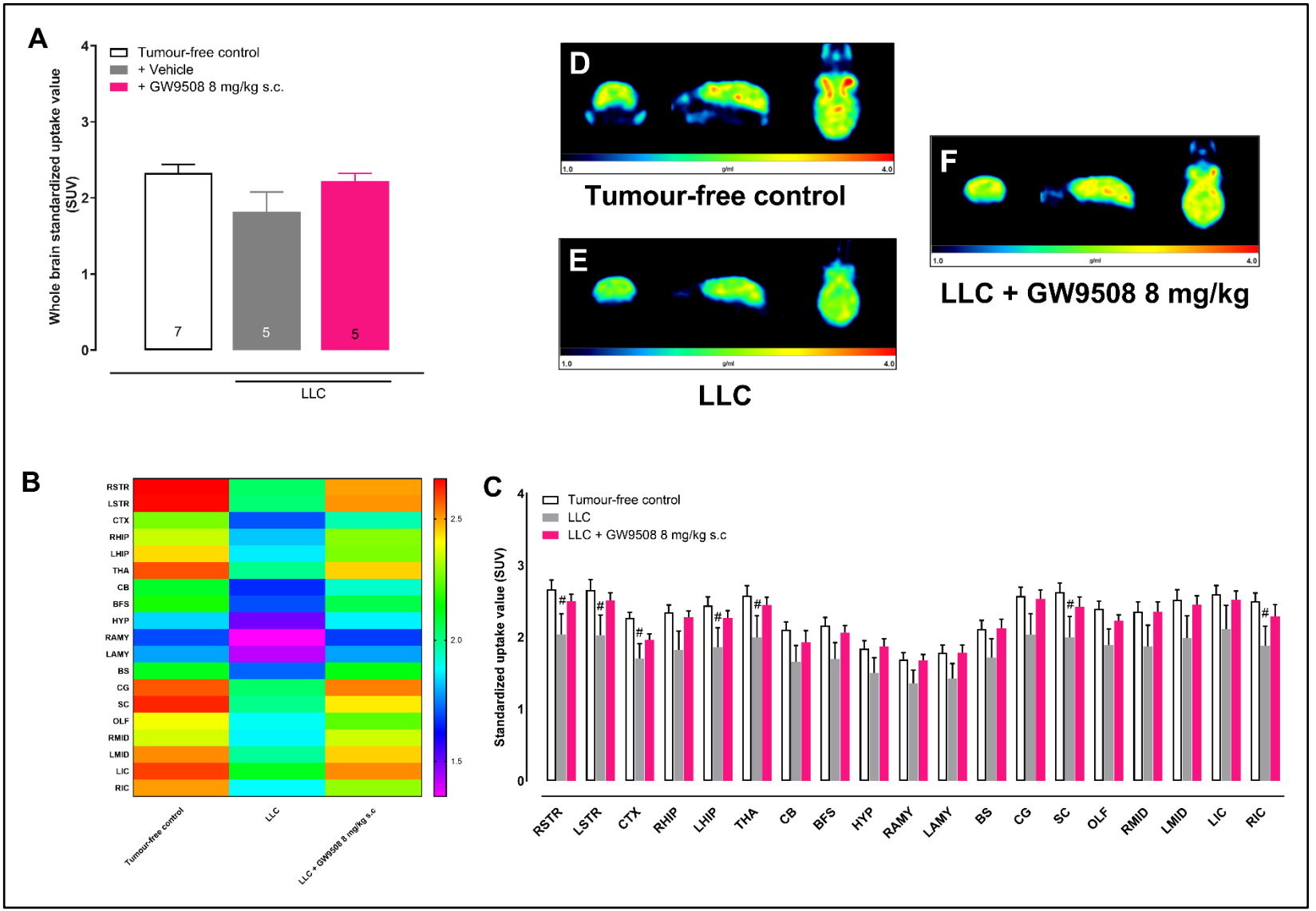

A
Tumour-free control
+ Vehicle
+ GW9508 8 mg/kg s.c.
Whole brain standardized uptake value (SUV)
4
3
2
1
0
7
5
5
LLC
D
Tumour-free control
E
LLC
F
LLC + GW9508 8 mg/kg
1.0
g/ml
4.0
B
RSTR
LSTR
CTX
RHIP
LHIP
THA
CB
BFS
HYP
RAMY
LAMY
BS
CG
SC
OLF
RMID
LMID
LIC
RIC
2.5
2.0
1.5
Tumour-free control
LLC
LLC + GW9508 8 mg/kg s.c
C
Tumour-free control
LLC
LLC + GW9508 8 mg/kg s.c
Standardized uptake value (SUV)
4
3
2
1
0
#
RSTR
LSTR
CTX
RHIP
LHIP
THA
CB
BFS
HYP
RAMY
LAMY
BS
CG
SC
OLF
RMID
LMID
LIC
RIC

Fig. 14 Effects of the repeated treatment with GW9508 (8 mg/kg, s.c.) on the [18F]-FDG brain hypometabolism associated to cancer-induced cachexia. (A) Whole brain metabolism evaluation after 21 days of tumour implantation. The panels B and C show the glucose uptake throughout different brain regions, as a heat map or in columns, respectively. Parameters are expressed as mean ± SEM. #P<0.05 denotes difference from control group. (Two-way ANOVA followed by Tukey's post hoc test). Representative images of (D) tumour-free control mice, (E) LLC-bearing mice, and (F) cancer cachectic mice treated with GW9508 (8 mg/kg, s.c.). Abbreviations: RSTR, right striatum region; LSTR, left striatum region; CTX, cortex; RHIP, right hippocampal region; LHIP, left hippocampal region; THA, thalamus; CB, cerebellum; BFS, basal forebrain/septum; HYP, hypothalamus; RAMY, right amygdala; LAMY, left amygdala; BS, brainstem; CG, cingulate gyrus; SC, superior colliculus; OLF, olfactory areas; RMID, right midbrain; LMID, left midbrain; LIC, left inferior colliculus; RIC, right inferior colliculus.

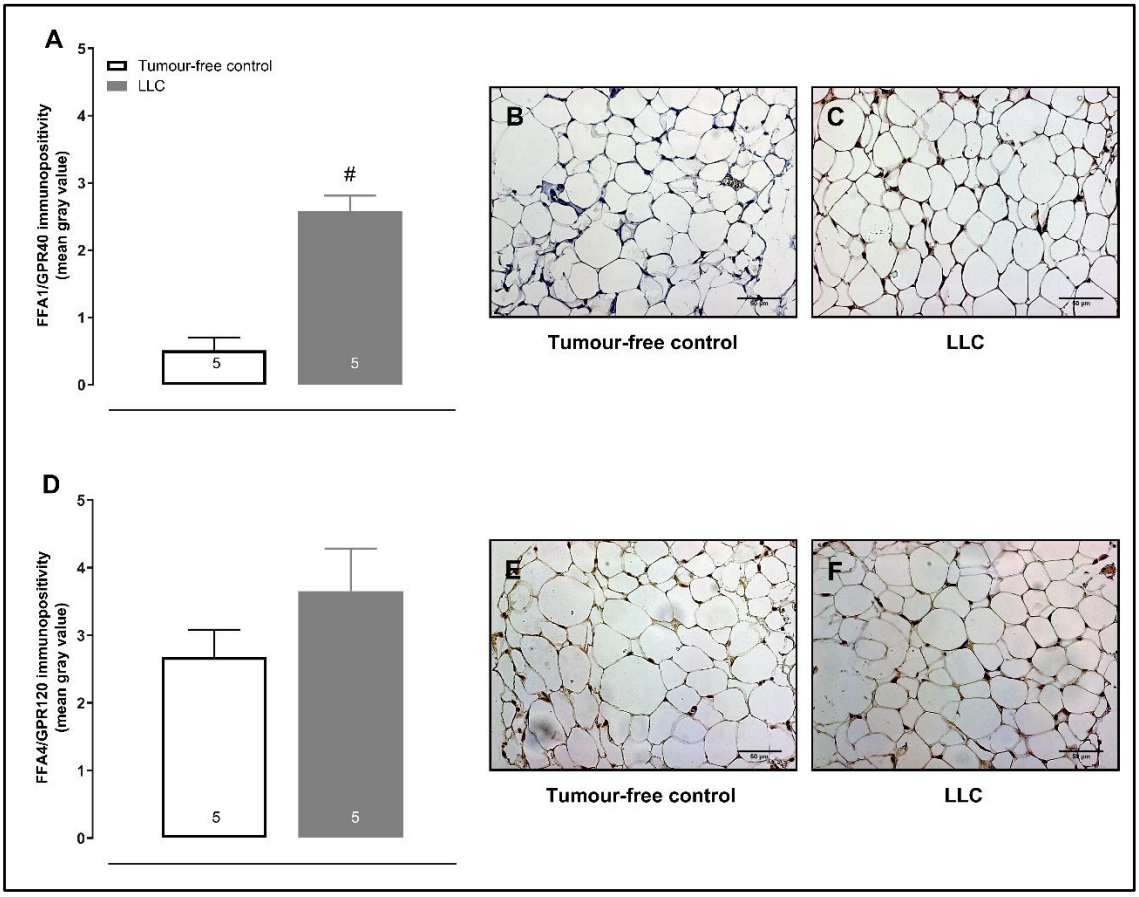


Fig. 15 FFA1 and FFA4 receptor immunopositivity in subcutaneous adipose tissue of LLC-bearing mice. (A) FFA1 receptor and (D) FFA4 receptor immunopositivity (expressed as mean grey value) in isWAT from tumour-free control and LLC-bearing mice. #P<0.05 denotes difference from control group. (B, C, E and F) Representative images of FFA1 and FFA4 immunolabelling in isWAT from tumour-free control and LLC-injected mice (One-way ANOVA followed by Bonferroni's post hoc test).

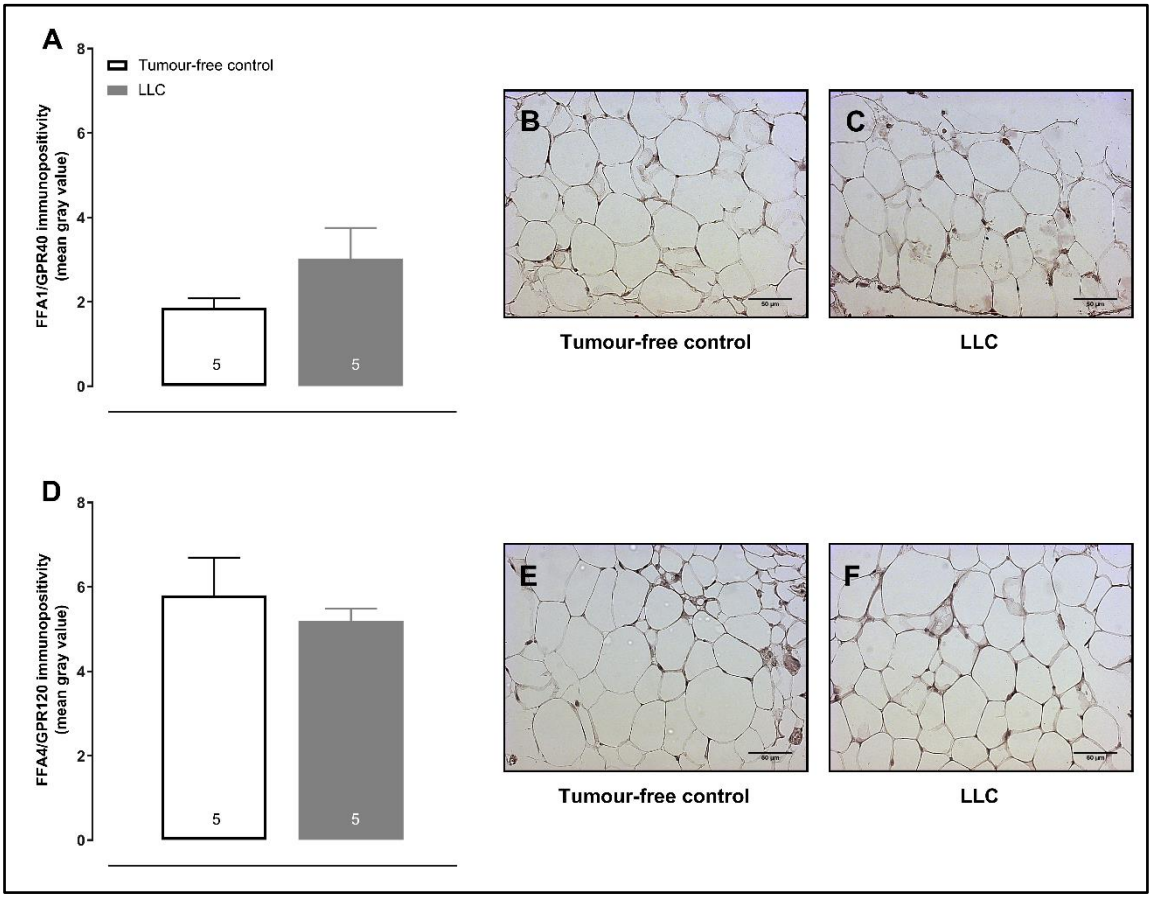


Fig. 16 FFA1 and FFA4 receptor immunopositivity in visceral adipose tissue of LLC-bearing mice. (A) FFA1 receptor and (D) FFA4 receptor immunopositivity (expressed as mean grey value) in epWAT from tumour-free control and LLC-bearing mice. (B, C, E and F) Representative images of FFA1 and FFA4 immunolabelling in epWAT from tumour-free control and LLC-injected mice.

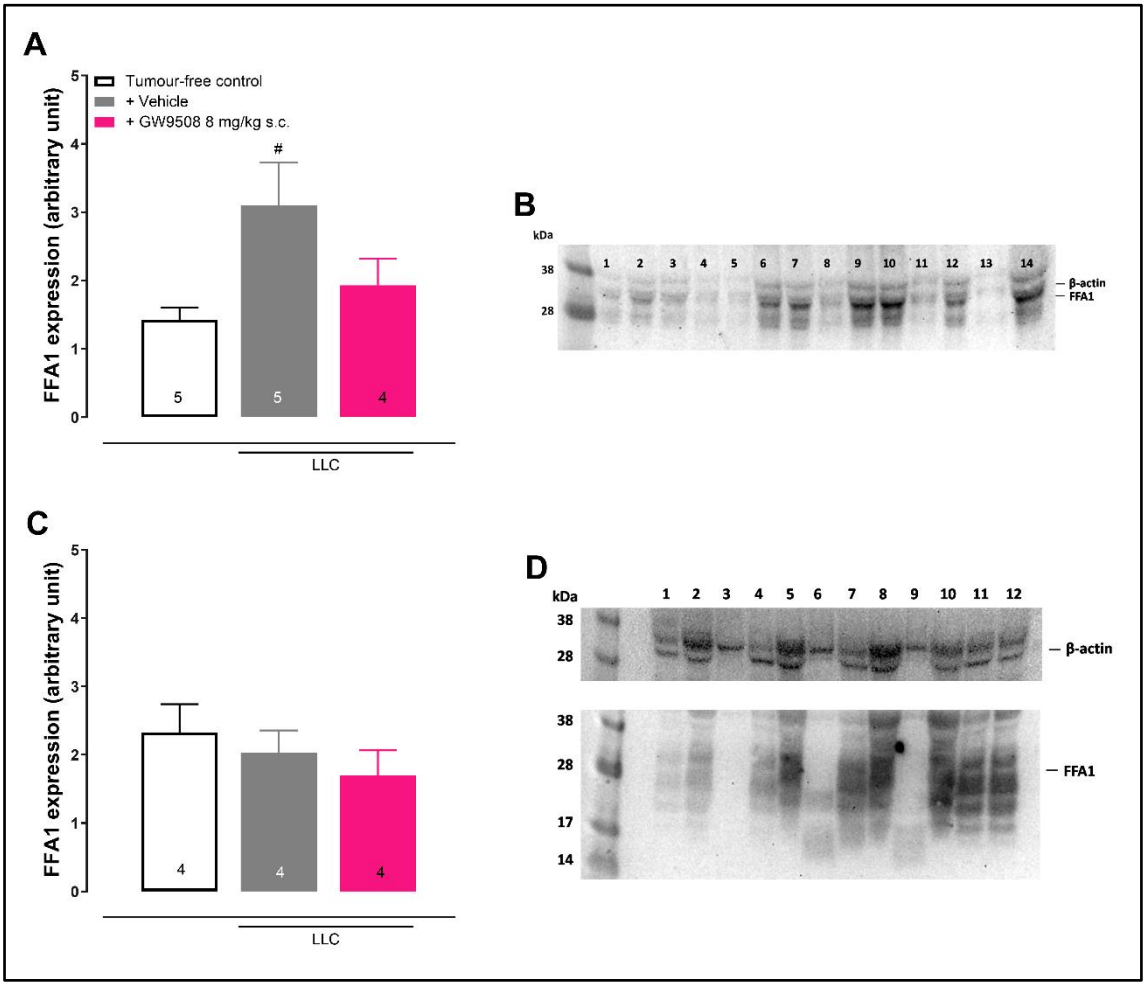
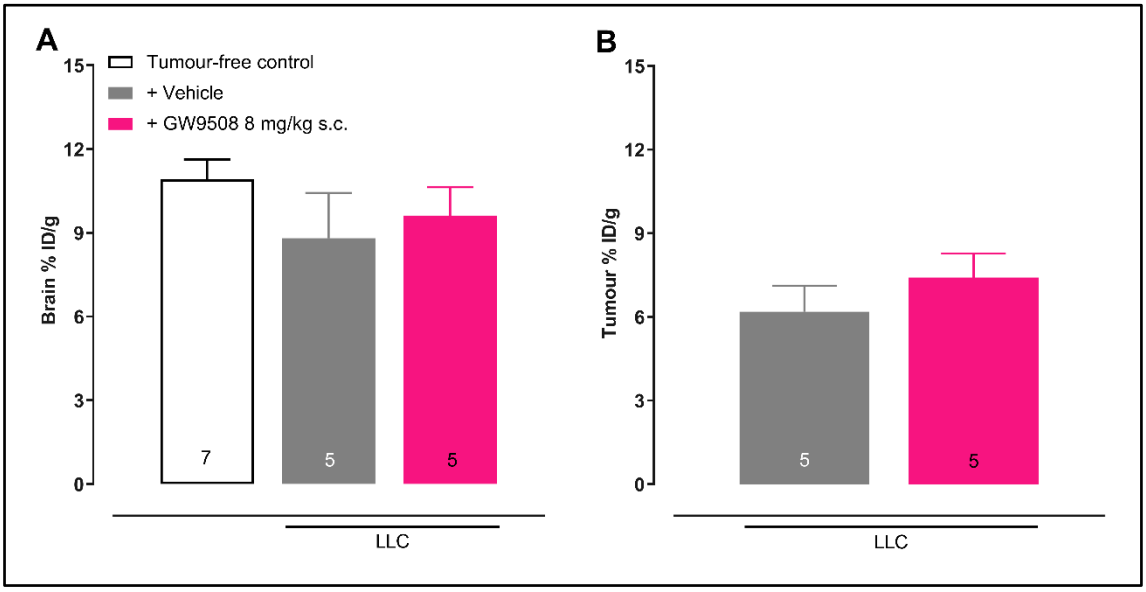


Fig. 17 Effects of the repeated treatment with GW9508 (8 mg/kg, s.c.) on FFA1 protein expression and their representative blots in gastrocnemius muscle and pancreas from LLC-bearing mice. (A) Evaluation of FFA1 protein expression in the gastrocnemius muscle and (C) pancreas from LLC-bearing mice treated with GW9508, and their respective controls. (B) Representative blots from gastrocnemius shows: tumour-free control (lanes 1 to 5), LLC-injected (lanes 6-10) and LLC-bearing with GW9508 treatment (lanes 11-14). (D) Representative blots from pancreas shows: tumour-free control (lanes 1-4), LLC-bearing (5-8) and LLC-injected with GW9508 treatment (lanes 9-12). Parameters are expressed in mean±SEM. #P<0.05 denotes significance from tumour-free control group (One-way ANOVA followed by Bonferroni's post-hoc).



Supplementary Figure S1. Effects of [^{18}F]-FDG biodistribution in brain and tumour of LLC-bearing mice. The biodistribution of [^{18}F]-FDG tissue distribution was assessed in the (A) brain and in the (B) tumour GW9508-treated LLC bearing mice, and its respective controls. Parameters are expressed in mean±SEM.

References

1. WHO. Cancer fact sheet [Internet]. 2019 [cited 2019 Jun 10]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/cancer

2. Argilés JM, Stemmler B, López-Soriano FJ, Busquets S. Inter-tissue communication in cancer cachexia. Nat Rev Endocrinol [Internet]. 2018; Available from: http://dx.doi.org/10.1038/s41574-018-0123-0

3. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, *et al*. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol [Internet]. 2011 May [cited 2014 Jul 15];12(5):489–95. Available from: http://www.sciencedirect.com/science/article/pii/S1470204510702187

4. Von Haehling S, Anker SD. Cachexia as major underestimated unmet medical need: Facts and numbers. Int J Cardiol. 2012;161:121–3.

5. Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: understanding the molecular basis. Nat Rev Cancer [Internet]. 2014 Oct 9 [cited 2014 Oct 8];14(11):754–62. Available from: http://dx.doi.org/10.1038/nrc3829

6. Seelaender M, Laviano A, Busquets S, Püschel GP, Margaria T, Jr MLB. Inflammation in Cachexia. 2015;2015:2–4.

7. Kir S, White JP, Kleiner S, Kazak L, Cohen P, Baracos VE, *et al*. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature [Internet]. 2014;513(7516):100–4. Available from: http://dx.doi.org/10.1038/nature13528

8. Mirza KA, Tisdale MJ. Role of Ca 2 + in proteolysis-inducing factor (PIF) - induced atrophy of skeletal muscle. Cell Signal [Internet]. 2012;24(11):2118–22. Available from: http://dx.doi.org/10.1016/j.cellsig.2012.07.016

9. Tsoli M, Swarbrick MM, Robertson GR. Lipolytic and thermogenic depletion of adipose tissue in cancer cachexia. Semin Cell Dev Biol [Internet]. 2015;1–14. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1084952115002463

10. Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated cachexia. Nat Rev Dis Prim [Internet]. 2018;4:1–18. Available from: http://dx.doi.org/10.1038/nrdp.2017.105

11. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients. 2016;8:1–17.

12. Serhan CN, Chiang N, Dalli J, Levy BD. Lipid Mediators in the Resolution of Inflammation. Cold Spring Harb Perspect Biol. 2015;7.

13. Lavriv DS, Neves PM, Ravasco P. Should omega-3 fatty acids be used for adjuvant treatment of cancer cachexia? Clin Nutr ESPEN [Internet]. 2018;25:18–25. Available from: https://doi.org/10.1016/j.clnesp.2018.02.006

14. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, *et al*. ESPEN guidelines on nutrition in cancer patients. Clin Nutr [Internet]. 2017;36(1):11–48. Available from: http://dx.doi.org/10.1016/j.clnu.2016.07.015

15. Freitas RD, Campos MM. Protective Effects of Omega-3 Fatty Acids in Cancer-Related Complications. Nutrients. 2019;11(945):1–23.

16. Briscoe CP, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, et al. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem [Internet]. 2003 Mar 28 [cited 2014 May 23];278(13):11303–11. Available from: http://www.jbc.org/content/278/13/11303.long

17. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med [Internet]. 2005;11(1):90–4. Available from: http://www.nature.com/doifinder/10.1038/nm1168

18. Sona C, Kumar A, Dogra S, Arun B, Umrao D. Docosahexaenoic acid modulates brain-derived neurotrophic factor via GPR40 in the brain and alleviates diabesity-associated learning and memory deficits in mice. Neurobiol Dis [Internet]. 2018;118(June):94–107. Available from: https://doi.org/10.1016/j.nbd.2018.07.002

19. Yang Y, Tian X, Xu D, Zheng F, Lu X, Zhang Y, et al. GPR40 modulates epileptic seizure and NMDA receptor function. Sci Adv. 2018;(October):1–12.

20. Liu H Da, Wang WB, Xu ZG, Liu CH, He DF, Du LP, et al. FFA4 receptor (GPR120): A hot target for the development of anti-diabetic therapies. Eur J Pharmacol [Internet]. 2015;763:160–8. Available from: http://dx.doi.org/10.1016/j.ejphar.2015.06.028

21. Freitas RDS, Costa KM, Nicoletti NF, Kist LW, Bogo MR, Campos MM. Omega-3 fatty acids are able to modulate the painful symptoms associated to cyclophosphamide-induced-hemorrhagic cystitis in mice. J Nutr Biochem [Internet]. 2016 Jan [cited 2015 Dec 30];27:219–32. Available from: http://www.sciencedirect.com/science/article/pii/S0955286315002429

22. Aizawa F, Nakamoto K, Tokuyama S. The involvement of free fatty acid-GPR40 / FFAR1 signaling in chronic social defeat stress-induced pain prolongation in C57BL / 6J male mice. Psychopharmacology (Berl). 2018;1:2335–47.

23. Nishinaka T, Yamashita T, Nakamoto K, Kasuya F, Tokuyama S. Involvement of the Long-Chain Fatty Acid Receptor GPR40 in Depression-Related Behavior. J Pharmacol Sci. 2014;115:112–5.

24. Khan MZ, Zhuang X, He L. GPR40 receptor activation leads to CREB phosphorylation and improves cognitive performance in an Alzheimer's disease mouse model. Neurobiol Learn Mem [Internet]. 2016;131:46–55. Available from: http://dx.doi.org/10.1016/j.nlm.2016.03.006

25. Oliveira V, Marinho R, Vitorino D, Santos GA, Moraes JC, Dragano N, et al. Diets containing α-linolenic (ω3) or oleic (ω9) fatty acids rescues obese mice from insulin resistance. Endocrinology. 2015;156(11):4033–46.

26. Dátilo MN, Ramos M, Formigari G, Rodrigues P, de Moura L, da Silva A, et al. Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor. Sci Rep. 2018;8(September):1–13.

27. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. Cell [Internet]. 2010;142(5):687–98. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867410008883

28. Mancini AD, Poitout V. The fatty acid receptor FFA1/GPR40 a decade later: How much do we know? Trends Endocrinol Metab [Internet]. 2013;24(8):398–407. Available from: http://dx.doi.org/10.1016/j.tem.2013.03.003

29. Moura-Assis A, Afonso MS, de Oliveira V, Morari J, dos Santos GA, Koike M, et al. Flaxseed oil rich in omega-3 protects aorta against inflammation and endoplasmic reticulum stress partially mediated by GPR120 receptor in obese, diabetic and dyslipidemic mice models. J Nutr Biochem [Internet]. 2018;53:9–19. Available from: https://doi.org/10.1016/j.jnutbio.2017.09.015

30. Gaspar R, Veiga C, Bessi M, Dátilo M, Sant'Ana M, Rodrigues P, et al. Unsaturated fatty acids from flaxseed oil and exercise modulate GPR120 but not GPR40

in the liver of obese mice : a new anti-inflammatory approach. J Nutr Biochem [Internet]. 2019;66:52–62. Available from: https://doi.org/10.1016/j.jnutbio.2018.12.003

31. Ulven T, Christiansen E. Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. Annu Rev Nutr [Internet]. 2015;35:239–63. Available from: http://www.annualreviews.org/doi/abs/10.1146/annurev-nutr-071714-034410

32. Dragano NR V, Solon C, Ramalho AF, Moura RF De, Razolli DS, Christiansen E, et al. Polyunsaturated fatty acid receptors , GPR40 and GPR120 , are expressed in the hypothalamus and control energy homeostasis and inflammation. 2017;1–16.

33. Ma D, Tao B, Warashina S, Kotani S, Lu L, Kaplamadzhiev DB, et al. Expression of free fatty acid receptor GPR40 in the central nervous system of adult monkeys. Neurosci Res [Internet]. 2007 Aug [cited 2014 Jan 31];58(4):394–401. Available from: http://www.sciencedirect.com/science/article/pii/S0168010207001563

34. Hopkins MM, Meier KE. Free Fatty Acid Receptors and Cancer : From Nutrition to Pharmacology. Handb Exp Pharmacol. 2016;

35. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting : The ARRIVE Guidelines for Reporting Animal Research. Plos Biol. 2010;8(6):6–10.

36. Briscoe CP, Peat AJ, Mckeown SC, Corbett DF, Goetz AS, Littleton TR, et al. Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. Br J Pharmacol. 2006;148:619–28.

37. Hudson BD, Shimpukade B, Mackenzie AE, Butcher AJ, Pediani JD, Christiansen E, et al. The pharmacology of TUG-891, a potent and selective agonist of the free fatty acid receptor 4 (FFA4/GPR120), demonstrates both potential opportunity and possible

challenges to therapeutic agonism. Mol Pharmacol [Internet]. 2013;84(5):710–25. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3807074&tool=pmcentrez&rendertype=abstract

38. Sparks SM, Chen G, Collins JL, Danger D, Dock ST, Jayawickreme C, et al. Identification of diarylsulfonamides as agonists of the free fatty acid receptor 4 (FFA4/GPR120). Bioorganic Med Chem Lett [Internet]. 2014;24(14):3100–3. Available from: http://dx.doi.org/10.1016/j.bmcl.2014.05.012

39. Schiessel DL, Yamazaki RK, Kryczyk M, Coelho I, Yamaguchi AA, Pequito DCT, et al. α-Linolenic Fatty Acid Supplementation Decreases Tumor Growth and Cachexia Parameters in Walker 256 Tumor-Bearing Rats. Nutr Cancer. 2015;67(5):839–46.

40. Gong Z, Yoshimura M, Aizawa S, Kurotani R, Zigman JM, Sakai T, et al. G protein-coupled receptor 120 signaling regulates ghrelin secretion in vivo and in vitro. Am J Physiol Endocrinol Metab. 2014;12:28–35.

41. Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion N V, Peters JA, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2017/18:G protein-coupled receptors. Br J Pharmacol. 2017;174:17–129.

42. Silva RBM, Greggio S, Venturin GT, da Costa JC, Gomez MV, Campos MM. Beneficial Effects of the Calcium Channel Blocker CTK 01512-2 in a Mouse Model of Multiple Sclerosis. Mol Neurobiol. 2018;

43. Dagnino AP, Silva RBM, Chagastelles PC, Pereira TCB, Venturin GT, Greggio S, et al. Nociceptin/orphanin FQ receptor modulates painful and fatigue symptoms in a mouse model of fibromyalgia. Pain. 2019;

44. Bertelli JA, Mira JC. The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods. 1995;58(1–2):151–5.

45. Amaral MEA, Nery LR, Leite CE, Filgueira W, Junior DA, Campos MM. Pre-clinical effects of metformin and aspirin on the cell lines of different breast cancer subtypes. Invest New Drugs. 2018;36:782–96.

46. Penet MF, Gadiya MM, Krishnamachary B, Nimmagadda S, Pomper MG, Artemov D, et al. Metabolic signatures imaged in cancer-induced cachexia. Cancer Res. 2011;71(22):6948–56.

47. Bellaver B, Rocha AS, Souza DG, Leffa DT, De Bastiani MA, Schu G, et al. Activated peripheral blood mononuclear cell mediators trigger astrocyte reactivity. Brain Behav Immun. 2019;(May).

48. Su X, Cheng K, Jeon J, Shen B, Venturin GT, Hu X, et al. Comparison of Two Site-Specifically 18F-Labeled Affibodies for PET Imaging of EGFR Positive Tumors. Mol Pharm. 2014;11:3947–56.

49. Harada S, Haruna Y, Aizawa F, Matsuura W, Nakamoto K. Involvement of GPR40 , a long-chain free fatty acid receptor , in the production of central post-stroke pain after global cerebral ischemia. Eur J Pharmacol [Internet]. 2014;744:115–23. Available from: http://dx.doi.org/10.1016/j.ejphar.2014.09.036

50. Dupré A, Malik HZ. Inflammation and cancer: What a surgical oncologist should know. Eur J Surg Oncol. 2018;44(5):566–70.

51. Dempersmier J, Sul HS. Shades of brown: a model for thermogenic fat. Front Endocrinol (Lausanne). 2015;6(May):1–6.

52. Hanai N, Terada H, Hirakawa H, Suzuki H, Nishikawa D, Beppu S, et al. Prospective randomized investigation implementing immunonutritional therapy using a

nutritional supplement with a high blend ratio of ω-3 fatty acids during the perioperative period for head and neck carcinomas. Jpn J Clin Oncol. 2018;48(4):356–61.

53. Abe K, Uwagawa T, Haruki K, Takano Y. Effects of ω-3 Fatty Acid Supplementation in Patients with Bile Duct or Pancreatic Cancer Undergoing Chemotherapy. Anticancer Res. 2018;2375:2369–75.

54. He F, Lope MA, Fr FO, Pamela K. Toll-Like Receptor-4 Disruption Suppresses Adipose Tissue Remodeling and Increases Survival in Cancer Cachexia Syndrome. Sci Rep. 2018;8(November):1–14.

55. Chen X, Wu Y, Yang T, Wei M, Wang Y, Deng X, et al. Salidroside alleviates cachexia symptoms in mouse models of cancer cachexia via activating mTOR signalling. J Cachexia Sarcopenia Muscle. 2016;7(January):224–32.

56. Carnier M, Silva FP, Miranda DA De, Hachul CL, Rischiteli A, Neto NIP, et al. Diet Supplemented with Chia Flour did not Modified the Inflammatory Process and Tumor Development in Wistar Rats Inoculated Walker 256 Cells. Nutr Cancer [Internet]. 2018;0(0):1–10. Available from: https://doi.org/10.1080/01635581.2018.1502329

57. Hajjaji N, Couet C, Besson P, Bougnoux P. DHA Effect on Chemotherapy-Induced Body Weight Loss : An Exploratory Study in a Rodent Model of Mammary Tumors. Nutr Cancer. 2012;61(7):37–41.

58. Deval C, Capel F, Laillet B, Polge C, Béchet D, Taillandier D, et al. Docosahexaenoic acid-supplementation prior to fasting prevents muscle atrophy in mice. J Cachexia Sarcopenia Muscle. 2016;7(5):587–603.

59. Pappalardo G, Almeida A, Ravasco P. Eicosapentaenoic acid in cancer improves body composition and modulates metabolism. Nutrition [Internet]. 2015 Apr [cited 2015 Aug 24];31(4):549–55. Available from: http://www.sciencedirect.com/science/article/pii/S0899900714005012

60. Gorjao R, Miguel C, Duarte T, Serdan A, Leonardo V, Diniz S, et al. New insights on the regulation of cancer cachexia by N-3 polyunsaturated fatty acids. Pharmacol Ther [Internet]. 2018;(xxxx). Available from: https://doi.org/10.1016/j.pharmthera.2018.12.001

61. Kita T, Kadochi Y, Takahashi K, Fukushima K, Yamasaki E, Uemoto T, et al. Diverse effects of G-protein-coupled free fatty acid receptors on the regulation of cellular functions in lung cancer cells. Exp Cell Res [Internet]. 2016;342(2):193–9. Available from: http://dx.doi.org/10.1016/j.yexcr.2016.03.008

62. Chu X, Zhou Q, Xu Y, Jiang J, Li Q, Zhou Q, et al. Aberrant fatty acid profile and FFAR4 signaling confer endocrine resistance in breast cancer. J Exp Clin Cancer Res. 2019;38(1):1–18.

63. Zhao Y, Zhang L, Yan A, Chen D, Xie R, Liu Y, et al. Grifolic acid induces GH3 adenoma cell death by inhibiting ATP production through a GPR120-independent mechanism. BMC Pharmacol Toxicol. 2018;19(1):1–9.

64. Hopkins MM, Meier KE. Free fatty acid receptor (FFAR) agonists inhibit proliferation of human ovarian cancer cells. Prostaglandins Leukot Essent Fat Acids [Internet]. 2017;122(December 2016):24–9. Available from: http://dx.doi.org/10.1016/j.plefa.2017.06.013

65. Kandarian SC, Nosacka RL, Delitto AE, Judge AR, Judge SM, Ganey JD, et al. Tumour-derived leukaemia inhibitory factor is a major driver of cancer cachexia and morbidity in C26 tumour-bearing mice. 2018;(September):1109–20.

66. Gotoh C, Hong Y, Iga T, Hishikawa D, Suzuki Y, Song S, et al. The regulation of adipogenesis through GPR120. Biochem Biophys Res Commun. 2007;354:591–7.

67. Mckenzie C V, Colonne CK, Fraser ST. Splenomegaly : Pathophysiological bases and therapeutic options. Int J Biochem Cell Biol. 2018;94:40–3.

68. Toledo M, Penna F, Busquets S, López-Soriano FJ, Argilés JM. Distinct behaviour of sorafenib in experimental cachexia-inducing tumours: the role of STAT3. PLoS One [Internet]. 2014;9(12):e113931. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4250056&tool=pmcentrez&rendertype=abstract

69. Zhang X, Zhang F, Qiao W, Zhang X, Zhao Z, Li M. Low Hematocrit Is a Strong Predictor of Poor Prognosis in Lung Cancer Patients. Biomed Res Int. 2018;

70. Huang TH, Chiu YH, Chan YL, Chiu YH, Wang H, Huang KC, et al. Prophylactic administration of fucoidan represses cancer metastasis by inhibiting vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) in Lewis tumor-bearing mice. Mar Drugs. 2015;13(4):1882–900.

71. Khadge S, Thiele GM, Graham J, Timothy S, Lynell RM, Black PN, et al. Long-chain omega-3 polyunsaturated fatty acids decrease mammary tumor growth , multiorgan metastasis and enhance survival. Clin Exp Metastasis [Internet]. 2018;35(8):797–818. Available from: http://dx.doi.org/10.1007/s10585-018-9941-7

72. Arora GK, Iyengar P, Infante RE, Arora GK, Gupta A, Narayanan S, et al. Cachexia-associated adipose loss induced by tumor-secreted leukemia inhibitory factor is counterbalanced by decreased leptin Graphical abstract Find the latest version : Cachexia-associated adipose loss induced by tumor-secreted leukemia inhibitory factor. J Clin Investig Insight. 2018;3(14).

73. Shirai Y, Okugawa Y, Hishida A, Ogawa A, Okamoto K. Fish oil-enriched nutrition combined with systemic chemotherapy for gastrointestinal cancer patients with cancer cachexia. Sci Rep. 2017;(November 2016):1–9.

74. Baker LA, Martin NRW, Kimber MC, Lewis MP. Resolvin E1 (RvE1) attenuates LPS induced inflammation and subsequent atrophy in C2C12 myotubes. J Cell Biochem. 2018;(February):6094–103.

75. Brown JL, Lee DE, Rosa-caldwell ME, Brown LA, Perry RA, Haynie WS, et al. Protein imbalance in the development of skeletal muscle wasting in tumour-bearing mice. J Cachexia Sarcopenia Muscle. 2018;(October):987–1002.

76. Folker ES, Baylies MK. Nuclear positioning in muscle development and disease. Front Physiol. 2013;4(December):1–10.

77. Judge SM, Nosacka RL, Delitto D, Gerber MH, Cameron ME, Trevino JG, et al. Skeletal Muscle Fibrosis in Pancreatic Cancer Patients with Respect to Survival. JNCI Cancer Spectr. 2019;2(3):1–13.

78. Inaba S, Hinohara A, Id MT, Tsujikawa K. Muscle regeneration is disrupted by cancer cachexia without loss of muscle stem cell potential. PLoS One. 2018;1–15.

79. Chevalier S, Farsijani S. Cancer cachexia and diabetes: similarities in metabolic alterations and possible treatment(.). Appl Physiol Nutr Metab. 2014;

80. Watterson KR, Hudson BD, Ulven T, Milligan G. Treatment of type 2 diabetes by free Fatty Acid receptor agonists. Front Endocrinol (Lausanne) [Internet]. 2014 Jan 28 [cited 2014 Nov 5];5:137. Available from: http://journal.frontiersin.org/Journal/10.3389/fendo.2014.00137/abstract

81. Holliday ND, Watson S, Brown AJH, Smith NJ, Cardiac VC. Drug discovery opportunities and challenges at G protein coupled receptors for long chain free fatty acids. Front Endocrinol (Lausanne). 2012;2(January):1–12.

82. Satapati S, Qian Y, Wu MS, Petrov A, Dai G, Wang S, et al. GPR120 suppresses adipose tissue lipolysis and synergizes with GPR40 in antidiabetic efficacy. J Lipid Res. 2017;58.

83. Das SK, Eder S, Schauer S, Diwoky C, Temmel H, Guertl B, et al. Adipose Triglyceride Lipase Contributes to Cancer-Associated Cachexia. Science (80-). 2013;233(2011).

84. Mann A, Thompson A, Robbins N, Blomkalns AL. Localization , Identification , and Excision of Murine Adipose Depots. J Vis Exp. 2014;(December):1–7.

85. Michaelis KA, Zhu X, Burfeind KG, Krasnow SM, Levasseur PR, Morgan TK, et al. Establishment and characterization of a novel murine model of pancreatic cancer cachexia. J Cachexia Sarcopenia Muscle. 2017;8(July):824–38.

86. Parmar MP, Vanderbyl BL, Kanbalian M, Windholz TY, Tran A, Jagoe RT. A multidisciplinary rehabilitation programme for cancer cachexia improves quality of life. BMJ Support Palliat Care. 2017;7:441–9.

87. Wallengren O, Lundholm K, Bosaeus I. Diagnostic criteria of cancer cachexia : relation to quality of life , exercise capacity , and survival in unselected palliative care patients. Support Care Cancer. 2013;21:1569–77.

88. Campos CA, Bowen AJ, Han S, Wisse BE, Palmiter RD, Schwartz MW. Cancer-induced anorexia and malaise are mediated by CGRP neurons in the parabrachial nucleus. Nat Neurosci. 2017;20(7):934–42.

89. Iwata Y, Suzuki N, Ohtake H, Kamauchi S, Hashimoto N, Kiyono T, et al. Cancer cachexia causes skeletal muscle damage via transient receptor potential vanilloid 2-independent mechanisms, unlike muscular dystrophy. J Cachexia Sarcopenia Muscle. 2016;(November 2015):366–76.

90. Nakamoto K, Nishinaka T, Sato N, Mankura M, Koyama Y, Kasuya F, et al. Hypothalamic GPR40 Signaling Activated by Free Long Chain Fatty Acids Suppresses CFA-Induced Inflammatory Chronic Pain. Price TJ, editor. PLoS One [Internet]. 2013

Jan [cited 2014 Jan 15];8(12):e81563. Available from: http://dx.plos.org/10.1371/journal.pone.0081563

91. Aizawa F, Nishinaka T, Yamashita T, Nakamoto K, Kurihara T, Hirasawa A, et al. GPR40/FFAR1 deficient mice increase noradrenaline levels in the brain and exhibit abnormal behavior. J Pharmacol Sci [Internet]. 2016;132(4):249–54. Available from: http://dx.doi.org/10.1016/j.jphs.2016.09.007

92. Aizawa F, Ogaki Y, Kyoya N, Nishinaka T, Nakamoto K, Kurihara T, et al. The Deletion of GPR40/FFAR1 Signaling Damages Maternal Care and Emotional Function in Female Mice. Biol Pharm Bull [Internet]. 2017;40(8):1255–9. Available from: https://www.jstage.jst.go.jp/article/bpb/40/8/40_b17-00082/_article

93. Auguste S, Fisette A, Fernandes MF, Hryhorczuk C, Poitout V, Alquier T, et al. Central Agonism of GPR120 Acutely Inhibits Food Intake and Food Reward and Chronically Suppresses Anxiety-Like Behavior in Mice. Int J Neuropsychopharmacol. 2016;19(7):1–10.

94. Cintra D, Ropelle E, Moraes J, Pauli J, Morari J, de Souza C, et al. Unsaturated Fatty Acids Revert Diet-Induced Hypothalamic Inflammation in Obesity. PLoS One. 2012;7(1).

95. Pin F, Busquets S, Toledo M, Camperi A, Lopez-Soriano FJ, Costelli P, et al. Combination of exercise training and erythropoietin prevents cancer-induced muscle alterations. Oncotarget [Internet]. 2015;6(41):43202–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26636649

96. Murphy KT, Struk A, Malcontenti-Wilson C, Christophi C, Lynch GS. Physiological characterization of a mouse model of cachexia in colorectal liver metastases. Am J Physiol Regul Integr Comp Physiol [Internet]. 2013;304(10):R854-64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23485871

97. Mauricio AF, Minatel E, Neto HS, Marques MJ. Effects of fish oil containing eicosapentaenoic acid and docosahexaenoic acid on dystrophic mdx mice. Clin Nutr [Internet]. 2013;32(4):636–42. Available from: http://dx.doi.org/10.1016/j.clnu.2012.11.013

98. Kim N, Lee JO, Lee HJ, Kim HI, Kim JK, Lee YW, et al. Endogenous ligand for GPR120, docosahexaenoic acid, exerts benign metabolic effects on the skeletal muscles via AMP-activated protein kinase pathway. J Biol Chem. 2015;290(33):20438–47.

99. Konsman JP, Blomqvist A. Forebrain patterns of c-Fos and FosB induction during cancer-associated anorexia – cachexia in rat. Eur J Neurosci. 2005;21(March):2752–66.

100. Zhang W, Ning N, Li X, Niu G, Bai L, Guo Y. Changes of Brain Glucose Metabolism in the Pretreatment Patients with Non-Small Cell Lung Cancer: A Retrospective PET / CT Study. PLoS One. 2016;1–10.

101. Khan MZ, He L. The role of polyunsaturated fatty acids and GPR40 receptor in brain. Neuropharmacology [Internet]. 2017;113(24):639–51. Available from: http://dx.doi.org/10.1016/j.neuropharm.2015.05.013

102. Falomir-Lockhart LJ, Cavazzutti GF, Giménez E, Toscani AM, Salvador GA, Nacional U. Fatty Acid Signaling Mechanisms in Neural Cells: Fatty Acid Receptors. Front Cell Neurosci. 2019;13(April):1–24.

103. Moriyama R, Deura C, Imoto S, Nose K, Fukushima N. Expression of the long-chain fatty acid receptor GPR120 in the gonadotropes of the mouse anterior pituitary gland. Histochem Cell Biol. 2015;143:21–7.

104. Yin J, Li H, Meng C, Chen D, Chen Z, Wang Y, et al. Inhibitory effects of omega-3 fatty acids on early brain injury after subarachnoid hemorrhage in rats : Possible involvement of G binding protein-1 signaling pathway. Int J Biochem Cell Biol [Internet]. 2016;75:11–22. Available from: http://dx.doi.org/10.1016/j.biocel.2016.03.008

105. Mao X, Wu H, Tang X, Ali U, Liu H, Wang Y. Activation of GPR40 produces mechanical antiallodynia via the spinal glial interleukin- 10 / β -endorphin pathway. 2019;9:1–19.

106. Hara T, Kashihara D, Ichimura A, Kimura I, Tsujimoto G, Hirasawa A. Role of free fatty acid receptors in the regulation of energy metabolism. BBA - Mol Cell Biol Lipids [Internet]. 2014;1841(9):1292–300. Available from: http://dx.doi.org/10.1016/j.bbalip.2014.06.002

107. Oliveira V, Marinho R, Vitorino D, Santos GA, Moraes JC, Dragano N, et al. Diets containing alpha-linolenic (n-3) or oleic (n-9) fatty acids rescues obese mice from insulin resistance. Endocrinology. 2015;156(11):4033–46.

108. Philippe C, Wauquier F, Lyan B, Wittrant Y. GPR40 , a free fatty acid receptor , differentially impacts osteoblast behavior depending on differentiation stage and environment. Mol Cell Biochem. 2016;412:197–208.

109. Philippe C, Wauquier F, Leotoing L, Coxam V, Wittrant Y. GW9508, a free fatty acid receptor agonist, specifically induces cell death in bone resorbing precursor cells through increased oxidative stress from mitochondrial origin. Exp Cell Res [Internet]. 2013;319(19):3035–41. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23973666

110. Quesada-López T, Cereijo R, Turatsinze JV, Planavila A, Cairó M, Gavaldà-Navarro A, et al. The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. Nat Commun. 2016;7.

111. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human.

Nature [Internet]. 2012;483(7389):350–4. Available from: http://www.nature.com/doifinder/10.1038/nature10798

112. Kohara K, Obata A, Kimura T, Shimoda M, Moriuchi S, Okauchi S. Suppression of free fatty acid receptor 1 expression in pancreatic β-cells in obese type 2 diabetic db / db mice : a potential role of pancreatic and duodenal homeobox factor 1. Endocr J. 2019;66(1):43–50.

113. von Haehling S, Morley JE, Coats AJS, Anker SD. Ethical guidelines for publishing in the journal of cachexia, sarcopenia and muscle: update 2017. J Cachexia Sarcopenia Muscle. 2017;8(6):1081–3.

Capítulo 4

Perspectivas

- Realizar ensaios *in vitro* de co-cultura de atrofia muscular e de lipólise para analisar os efeitos dos agonistas e antagonistas dos receptores FFA1/FFA4.
- Realizar ensaios *in vitro* para avaliar os efeitos do meio condicionado pelas células LLC sobre macrófagos tratados com os ligantes dos receptores FFA1/FFA4.
- Investigar as vias de sinalização responsáveis pela resposta ao tratamento com o agonista dual de FFA1/FFA4, GW9508.
- Avaliar os efeitos da administração central do agonista GW9508 sobre o modelo de caquexia associada ao câncer.
- Avaliar os efeitos da administração central dos antagonistas do FFA1 e FFA4, GW1100 e AH7614, respectivamente, sobre o modelo de caquexia associada ao câncer, conjugados à administração central do agonista dual do FFA1/FFA4, GW9508.
- Avaliar o *sickness behavior* relacionado à caquexia associada ao câncer, após a administração central e periférica do agonista dual do FFA1/FFA4 e do antagonista seletivo do FFA1, GW1100, e do FFA4, AH7614.

Considerações Finais

O manejo nutricional como forma de tratamento adjacente às diferentes patologias é uma prática clínica comum. Todavia, o uso de nutrientes isolados para modulação dessa patologia é mais recente. Dentro desse raciocínio, existem duas linhas distintas, porém muito semelhantes: a imunonutrição e a farmaconutrição. A imunonutrição é caracterizada pelo uso de uma molécula específica a fim de modular uma resposta imunológica. A farmaconutrição, por outro lado, seria um efeito mais específico, de uma molécula advinda de um nutriente, agindo diretamente em um receptor. Dentro de ambas, podemos encaixar os ácidos graxos ômega-3, por agirem de ambas as maneiras.

Nos cuidados nutricionais de um paciente oncológico, o uso dos ácidos graxos ômega-3 é uma abordagem muito utilizada na prática clínica. Sabe-se que essas moléculas exercem efeitos benéficos aos pacientes, principalmente, no desenvolvimento do quadro caquético. Por outro lado, as diretrizes ainda questionam o uso, pois os estudos clínicos ainda se contradizem no que diz respeito ao efeito anti-caquético. De maneira interessante, não há uma grande quantidade de evidências científicas a respeito do uso dos ácidos graxos ômega-3 em outras complicações relacionadas ao câncer. Esses pacientes, além de comumente apresentarem caquexia, podem desenvolver depressão, apresentar dor e síndromes paraneoplásicas, que se caracterizam por uma grande quantidade de diferentes sintomas que podem ocorrer antes ou depois do diagnóstico. No segundo capítulo dessa Tese, foi realizada uma revisão de literatura que evidenciou a escassa quantidade de evidência clínica relacionando diretamente o uso dos ácidos graxos ômega-3 e essas outras complicações.

A caquexia associada ao câncer é considerada uma síndrome multifatorial que afeta um grande percentual dos pacientes oncológicos, não havendo cura. De maneira

importante, em torno de 80 % dos pacientes oncológicos desenvolvem caquexia, e 20 % destes chegam à óbito. A caquexia associada ao câncer afeta diretamente a qualidade de vida dos pacientes, por gerar inapetência, fadiga e perda de independência. O desenvolvimento do quadro caquético, no geral, também leva à uma pior resposta ao tratamento antineoplásico, consequentemente, aumentando o tempo de internação destes pacientes. Esse último fator demonstra que o cuidado nutricional do paciente oncológico é de extrema importância socioeconômica, uma vez que os custos de internação também aumentam em decorrência da desnutrição.

O uso dos ácidos graxos ômega-3, na forma de suplementação oral ou na forma de emulsões, é bastante corriqueiro na prática clínica no manejo de pacientes caquéticos. Todavia, as diretrizes atuais ainda levantam dúvidas a respeito da real eficácia do uso desse lipídeo. Por outro lado, há um número cada vez maior de evidências científicas demonstrando que metabólitos dos ácidos graxos ômega-3, como os mediadores pró-resolução, podem ser benéficos para o manejo de doenças. Um marco importante para a farmaconutrição foi o descobrimento de que receptores de membrana, acoplados à proteína G, são ativados e sensibilizados por lipídeos. Atualmente, sabe-se que essa família de receptores possui extrema importância na modulação metabólica e que surge como promissores alvos farmacológicos.

Os receptores de ácidos graxos, como o FFA1 e o FFA4, vêm sendo amplamente investigados como potenciais alvos farmacológicos para diabetes e obesidade. Porém, nenhuma molécula que ative esses receptores chegou ao mercado. De maneira interessante, de forma inédita, nosso trabalho mostra a modulação desses receptores em uma situação patológica semelhante a diabetes, porém, com sintomas clínicos muito distintos. Baseando-se nos nossos achados do trabalho experimento, pode-se inferir que a ativação desse receptor gera respostas diferentes dependendo do estado metabólico do

organismo. Ao mesmo tempo em que se observou, em outras publicações, que a ativação do FFA1 e FFA4 promovem diminuição do tecido adiposo e diminuição do peso corporal, em um quadro de obesidade, em uma situação como a caquexia, sua ativação parece ter uma ação lipogênica. Além do mais, vimos também, de maneira inédita, o receptor FFA1 sendo expresso no tecido adiposo de animais caquético, demonstrando que esse receptor, participa de alguma maneira no desenvolvimento da caquexia.

Levando em consideração de que a caquexia associada ao câncer, uma síndrome que afeta diferentes vias metabólicas e diferentes tecidos do organismo, ser tratada com apenas um alvo farmacológico é um tanto improvável. Os receptores de ácidos graxos livres, como o FFA1 e o FFA4, que são ativados pelos ácidos graxos ômega-3, surgem como alvos importantes pois, quando ativados ao mesmo tempo, em diferentes locais, podem potencializar seus efeitos, exercendo resultados benéficos ao organismo caquético.

Finalmente, podemos dizer que os ácidos graxos ômega-3 continuam sendo promissores no manejo do paciente oncológico, mesmo sendo pela ativação dos receptores FFA1 e FFA4, como pela modulação das vias pró-inflamatórias. Ademais, não existem dados na literatura demonstrando os efeitos dos mediadores pró-resolução, derivados dos ômega-3, na caquexia associada ao câncer. Além disso, a manipulação farmacológica do FFA1 e do FFA4 também surge no cenário no cuidado do paciente oncológico, abrindo novas portas para o estudo da farmaconutrição.

Referências

1. Dyerberg J, Bang HO, Hjorne N. Fatty acid composition of the plasma lipids in Greenland Eskimos. Am J Clin Nutr. 1975;28(9):958–66.
2. Bang HO, Dyerberg MD, Sinclair HM. The composition of the Eskimo western food in north. Am J Clin Nutr. 1980;33:2657–61.
3. Cholewski M, Tomczykowa M, Tomczyk M. A Comprehensive Review of Chemistry , Sources and Bioavailability of Omega-3 Fatty Acids. Nutrients. 2018;10(1662):1–33.
4. Sokoła-Wysoczańska E, Wysoczański T, Wagner J, Czyż K, Bodkowski R, Lochyński S, et al. Polyunsaturated Fatty Acids and Their Potential Therapeutic Role in Cardiovascular System Disorders—A Review. Nutrients. 2018;10(10):1561.
5. Simopoulos AP, DiNicolantonio JJ. The importance of a balanced ω-6 to ω-3 ratio in the prevention and management of obesity. Open Hear [Internet]. 2016;3(2):e000385. Available from: http://www.ncbi.nlm.nih.gov/pubmed/6362514%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/27843563%5Cnhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC5093368
6. Calder PC. Feeding the immune system. Proc Nutr Soc [Internet]. 2013 May 21 [cited 2013 May 22];(October 2012):1–11. Available from: http://www.journals.cambridge.org/abstract_S0029665113001286
7. Simopoulos AP. Evolutionary aspects of diet , the omega-6 / omega-3 ratio and genetic variation : nutritional implications for chronic diseases. Biomed Pharmacother. 2006;60:502–7.

8. Calder PC. Functional Roles of Fatty Acids and Their Effects on Human Health. J Parenter Enter Nutr. 2015;39(1):18–32.

9. Khadge S, Sharp JG, Mcguire TR, Thiele M, Black P, Dirusso C, et al. Immune regulation and anti-cancer activity by lipid in fl ammatory mediators. Int Immunopharmacol. 2018;65(October):580–92.

10. Serhan CN, Hong S, Gronert K, Colgan SP, Devchand PR, Mirick G, et al. Resolvins: A family of bioactive products of omega-3 fatty acid transformation circuits initiated by aspirin treatment that counter proinflammation signals. J Exp Med [Internet]. 2002 Oct 21 [cited 2013 May 24];196(8):1025–37. Available from: http://www.jem.org/cgi/doi/10.1084/jem.20020760

11. Serhan CN. Novel lipid mediators and resolution mechanisms in acute inflammation: to resolve or not? Am J Pathol [Internet]. 2010 Oct [cited 2013 May 24];177(4):1576–91. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2947253&tool=pmcentrez&rendertype=abstract

12. Muralidharan J, Papandreou C, Sala-Vila A, Rosique-Esteban N, Fitó M, Estruch R, et al. Fatty Acids Composition of Blood Cell Membranes and Peripheral Inflammation in the PREDIMED Study: A Cross-Sectional Analysis. Nutrients. 2019;11(3):576.

13. Serhan CN. Pro-resolving lipid mediators are leads for resolution physiology. Nature [Internet]. 2014 Jun 4 [cited 2014 Jun 4];510(7503):92–101. Available from: http://dx.doi.org/10.1038/nature13479

14. Serhan CN, Levy BD, Serhan CN, Levy BD. Resolvins in inflammation : emergence of the pro-resolving superfamily of mediators. J Clin Invest. 2018;128(7):2657–69.

15. Klein CP, Sperotto NDM, Maciel IS, Leite CE, Souza AH, Campos MM. Effects of D-series resolvins on behavioral and neurochemical changes in a fibromyalgia-like model in mice. Neuropharmacology [Internet]. 2014 Nov [cited 2014 Nov 6];86:57–66. Available from: http://www.sciencedirect.com/science/article/pii/S0028390814002196

16. Sulciner ML, Serhan CN, Gilligan MM, Mudge DK, Chang J, Gartung A, et al. Resolvins suppress tumor growth and enhance cancer therapy. J Exp Med. 2018;215(1):115–40.

17. Freitas RD, Campos MM. Protective Effects of Omega-3 Fatty Acids in Cancer-Related Complications. Nutrients. 2019;11(945):1–23.

18. Larrieu T, Layé S. Food for mood: Relevance of nutritional omega-3 fatty acids for depression and anxiety. Front Physiol. 2018;9:1–15.

19. Gammone MA, Riccioni G, Parrinello G, D'orazio N. Omega-3 polyunsaturated fatty acids: Benefits and endpoints in sport. Nutrients. 2019;11(1):1–16.

20. Briscoe CP, Tadayyon M, Andrews JL, Benson WG, Chambers JK, Eilert MM, et al. The orphan G protein-coupled receptor GPR40 is activated by medium and long chain fatty acids. J Biol Chem [Internet]. 2003 Mar 28 [cited 2014 May 23];278(13):11303–11. Available from: http://www.jbc.org/content/278/13/11303.long

21. Hirasawa A, Tsumaya K, Awaji T, Katsuma S, Adachi T, Yamada M, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through GPR120. Nat Med [Internet]. 2005;11(1):90–4. Available from: http://www.nature.com/doifinder/10.1038/nm1168

22. Alexander SPH, Christopoulos A, Davenport AP, Kelly E, Marrion N V, Peters JA, et al. THE CONCISE GUIDE TO PHARMACOLOGY 2017/18:G protein-

coupled receptors. Br J Pharmacol. 2017;174:17–129.

23. Tikhonova IG, Poerio E. Free fatty acid receptors: structural models and elucidation of ligand binding interactions. BMC Struct Biol [Internet]. 2015;15(1):16. Available from: http://www.biomedcentral.com/1472-6807/15/16

24. Miyamoto J, Hasegawa S, Kasubuchi M, Ichimura A, Nakajima A. Nutritional Signaling via Free Fatty Acid Receptors. Int J Mol Sci. 2016;17(450):1–12.

25. Tomita T, Hosoda K, Fujikura J, Inagaki N, Nakao K. The G-Protein-Coupled Long-Chain Fatty Acid Receptor GPR40 and Glucose Metabolism. Front Endocrinol (Lausanne) [Internet]. 2014;5(September):152. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4176464&tool=pmcentrez&rendertype=abstract

26. Khan MZ, He L. The role of polyunsaturated fatty acids and GPR40 receptor in brain. Neuropharmacology [Internet]. 2015; Available from: http://dx.doi.org/10.1016/j.neuropharm.2015.05.013

27. Yamashima T. Dual effects of the non-esterified fatty acid receptor ‘ GPR40 ’ for human health. Prog Lipid Res [Internet]. 2015;58:40–50. Available from: http://dx.doi.org/10.1016/j.plipres.2015.01.002

28. Itoh Y, Kawamata Y, Harada M, Kobayashi M, Fujii R, Fukusumi S, et al. Free fatty acids regulate insulin secretion from pancreatic beta cells through GPR40. Nature [Internet]. 2003 Mar 13 [cited 2013 Nov 13];422(6928):173–6. Available from: http://dx.doi.org/10.1038/nature01478

29. Briscoe CP, Peat AJ, Mckeown SC, Corbett DF, Goetz AS, Littleton TR, et al. Pharmacological regulation of insulin secretion in MIN6 cells through the fatty acid receptor GPR40: identification of agonist and antagonist small molecules. Br J Pharmacol. 2006;148:619–28.

30. Lan H, Hoos LM, Liu L, Tetzloff G, Hu W, Abbondanzo SJ, et al. Lack of FFAR1 / GPR40 Does Not Protect Mice From High-Fat Diet – Induced Metabolic Disease. 2008;57(November):2999–3006.

31. Matsuda-Nagasumi K, Takami-Esaki R, Iwachidow K, Yasuhara Y, Tanaka H, Ogi K, et al. Lack of GPR40/FFAR1 does not induce diabetes even under insulin resistance condition. Diabetes, Obes Metab. 2013;15:538–45.

32. Alquier T, Poitout V. GPR40: Good Cop, Bad Cop? Diabetes [Internet]. 2009;58(5):1035–6. Available from: http://diabetes.diabetesjournals.org/cgi/doi/10.2337/db09-0215

33. Yashiro H, Tsujihata Y, Takeuchi K, Hazama M, Johnson PR V, Rorsman P. The Effects of TAK-875 , a Selective G Protein-Coupled Receptor 40 / Free Fatty Acid 1 Agonist , on Insulin and Glucagon Secretion in Isolated Rat and Human Islets □. 2012;

34. Burant C, Viswanathan P, Marcinak J, Cao C, Vakilynejad M. TAK-875 versus placebo or glimepiride in type 2 diabetes mellitus: a phase 2, randomised, double-blind, placebo-controlled trial. Lancet. 2012;379:1430–11.

35. Sclafani A, Zukerman S, Ackroff K. GPR40 and GPR120 fatty acid sensors are critical for postoral but not oral mediation of fat preferences in the mouse. 2013;11210:1490–7.

36. Nakamoto K, Nishinaka T, Matsumoto K, Kasuya F, Mankura M, Koyama Y, et al. Involvement of the long-chain fatty acid receptor GPR40 as a novel pain regulatory system. Brain Res [Internet]. 2012 [cited 2014 Jan 28];1432:74–83. Available from: http://www.sciencedirect.com/science/article/pii/S0006899311020397

37. Fujita T, Matsuoka T, Honda T, Kabashima K, Hirata T, Narumiya S. A GPR40

agonist GW9508 suppresses CCL5, CCL17, and CXCL10 induction in keratinocytes and attenuates cutaneous immune inflammation. J Invest Dermatol [Internet]. 2011 Aug [cited 2014 Jan 31];131(8):1660–7. Available from: http://dx.doi.org/10.1038/jid.2011.123

38. Wang S, Awad KS, Elinoff JM, Dougherty EJ, Ferreyra G a., Wang JY, et al. G Protein-Coupled Receptor 40 (GPR40) and Peroxisome Proliferator-Activated Receptor γ (PPARγ): An Integrated Two-Receptor Signaling Pathway. J Biol Chem [Internet]. 2015;40(32):jbc.M115.638924. Available from: http://www.jbc.org/lookup/doi/10.1074/jbc.M115.638924

39. Edfalk S, Steneberg P, Edlund H. Gpr40 is expressed in enteroendocrine cells and mediates free fatty acid stimulation of incretin secretion. Diabetes. 2008;57(9):2280–7.

40. Nakamoto K, Nishinaka T, Ambo A, Mankura M, Kasuya F, Tokuyama S. Possible involvement of β-endorphin in docosahexaenoic acid-induced antinociception. Eur J Pharmacol [Internet]. 2011 [cited 2013 Dec 11];666(1):100–4. Available from: http://www.sciencedirect.com/science/article/pii/S0014299911006303

41. Freitas RDS, Costa KM, Nicoletti NF, Kist LW, Bogo MR, Campos MM. Omega-3 fatty acids are able to modulate the painful symptoms associated to cyclophosphamide-induced-hemorrhagic cystitis in mice. J Nutr Biochem [Internet]. 2016 Jan [cited 2015 Dec 30];27:219–32. Available from: http://www.sciencedirect.com/science/article/pii/S0955286315002429

42. Mao X, Wu H, Tang X, Ali U, Liu H, Wang Y. Activation of GPR40 produces mechanical antiallodynia via the spinal glial interleukin- 10 / β -endorphin pathway. 2019;9:1–19.

43. Khan MZ, He L. The role of polyunsaturated fatty acids and GPR40 receptor in

brain. Neuropharmacology. 2017.

44. Falomir-Lockhart LJ, Cavazzutti GF, Giménez E, Toscani AM, Salvador GA, Nacional U. Fatty Acid Signaling Mechanisms in Neural Cells: Fatty Acid Receptors. Front Cell Neurosci. 2019;13(April):1–24.

45. Aizawa F, Nakamoto K, Tokuyama S. The involvement of free fatty acid-GPR40 / FFAR1 signaling in chronic social defeat stress-induced pain prolongation in C57BL / 6J male mice. Psychopharmacology (Berl). 2018;1:2335–47.

46. Alavi MS, Shamsizadeh A, Azhdari-Zarmehri H, Roohbakhsh A. Orphan G protein-coupled receptors: The role in CNS disorders. Biomed Pharmacother [Internet]. 2018;98(December 2017):222–32. Available from: https://doi.org/10.1016/j.biopha.2017.12.056

47. Aizawa F, Nishinaka T, Yamashita T, Nakamoto K, Kurihara T, Hirasawa A, et al. GPR40/FFAR1 deficient mice increase noradrenaline levels in the brain and exhibit abnormal behavior. J Pharmacol Sci [Internet]. 2016;132(4):249–54. Available from: http://dx.doi.org/10.1016/j.jphs.2016.09.007

48. Aizawa F, Ogaki Y, Kyoya N, Nishinaka T, Nakamoto K, Kurihara T, et al. The Deletion of GPR40/FFAR1 Signaling Damages Maternal Care and Emotional Function in Female Mice. Biol Pharm Bull [Internet]. 2017;40(8):1255–9. Available from: https://www.jstage.jst.go.jp/article/bpb/40/8/40_b17-00082/_article

49. Yang Y, Tian X, Xu D, Zheng F, Lu X, Zhang Y, et al. GPR40 modulates epileptic seizure and NMDA receptor function. Sci Adv. 2018;(October):1–12.

50. Dragano NR V, Solon C, Ramalho AF, Moura RF De, Razolli DS, Christiansen E, et al. Polyunsaturated fatty acid receptors , GPR40 and GPR120 , are expressed in the hypothalamus and control energy homeostasis and inflammation. 2017;1–16.

51. Gorski JN, Pachanski MJ, Mane J, Plummer CW, Souza S, Thomas-Fowlkes BS, et al. GPR40 reduces food intake and body weight through GLP-1. Am J Physiol - Endocrinol Metab [Internet]. 2017;313(1):E37–47. Available from: http://ajpendo.physiology.org/lookup/doi/10.1152/ajpendo.00435.2016

52. Sona C, Kumar A, Dogra S, Arun B, Umrao D. Docosahexaenoic acid modulates brain-derived neurotrophic factor via GPR40 in the brain and alleviates diabesity-associated learning and memory deficits in mice. Neurobiol Dis [Internet]. 2018;118(June):94–107. Available from: https://doi.org/10.1016/j.nbd.2018.07.002

53. Li G, Robles S, Lu Z, Li Y, Krayer JW, Leite RS, et al. Upregulation of free fatty acid receptors in periodontal tissues of patients with metabolic syndrome and periodontitis. J Periodontal Res. 2018;(December):1–8.

54. Nakashima C, Shingo K, Tani RF, Luo YI, Kawahara I. Expression of long - chain fatty acid receptor GPR40 is associated with cancer progression in colorectal cancer : A retrospective study. 2018;8641–6.

55. Zhang K, Hu Z, Qi H, Shi Z, Chang Y, Yao Q. G-protein-coupled receptors mediate ω-3 PUFAs-inhibited colorectal cancer by activating the Hippo pathway. Oncotarget. 2016;7(36):58315–30.

56. Liotti A, Cosimato V, Mirra P, Calì G, Conza D, Secondo A, et al. Oleic acid promotes prostate cancer malignant phenotype via the G protein-coupled receptor FFA1 / GPR40. J Cell Physiol. 2018;(February):7367–78.

57. Liu H Da, Wang WB, Xu ZG, Liu CH, He DF, Du LP, et al. FFA4 receptor (GPR120): A hot target for the development of anti-diabetic therapies. Eur J Pharmacol [Internet]. 2015;763:160–8. Available from: http://dx.doi.org/10.1016/j.ejphar.2015.06.028

58. Ulven T, Christiansen E. Dietary Fatty Acids and Their Potential for Controlling Metabolic Diseases Through Activation of FFA4/GPR120. Annu Rev Nutr [Internet]. 2015;35:239–63. Available from: http://www.annualreviews.org/doi/abs/10.1146/annurev-nutr-071714-034410

59. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, et al. GPR120 Is an Omega-3 Fatty Acid Receptor Mediating Potent Anti-inflammatory and Insulin-Sensitizing Effects. Cell [Internet]. 2010;142(5):687–98. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0092867410008883

60. Abdel-magid AF. Therapeutic Potential of GPR120 Agonists for the Treatment of Type 2 Diabetes. 2015;1097–8.

61. Ichimura A, Hirasawa A, Poulain-Godefroy O, Bonnefond A, Hara T, Yengo L, et al. Dysfunction of lipid sensor GPR120 leads to obesity in both mouse and human. Nature [Internet]. 2012;483(7389):350–4. Available from: http://www.nature.com/doifinder/10.1038/nature10798

62. Rodriguez-Pacheco F, Garcia-Serrano S, Garcia-Escobar E, Gutierrez-Repiso C, Garcia-Arnes J, Valdes S, et al. Effects of obesity/fatty acids on the expression of GPR120. Mol Nutr Food Res [Internet]. 2014;58(9):1852–60. Available from: http://doi.wiley.com/10.1002/mnfr.201300666

63. Im D-S. Functions of omega-3 fatty acids and FFA4 (GPR120) in macrophages. Eur J Pharmacol [Internet]. 2015;4:1–8. Available from: http://linkinghub.elsevier.com/retrieve/pii/S0014299915004586

64. Auguste S, Fisette A, Fernandes MF, Hryhorczuk C, Poitout V, Alquier T, et al. Central Agonism of GPR120 Acutely Inhibits Food Intake and Food Reward and Chronically Suppresses Anxiety-Like Behavior in Mice. Int J Neuropsychopharmacol. 2016;19(7):1–10.

65. Quesada-López T, Cereijo R, Turatsinze JV, Planavila A, Cairó M, Gavaldà-Navarro A, et al. The lipid sensor GPR120 promotes brown fat activation and FGF21 release from adipocytes. Nat Commun. 2016;7.

66. Schilperoort M, van Dam AD, Hoeke G, Shabalina IG, Okolo A, Hanyaloglu AC, et al. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. EMBO Mol Med [Internet]. 2018;e8047. Available from: http://embomolmed.embopress.org/lookup/doi/10.15252/emmm.201708047

67. Senatorov IS, Moniri NH. The role of free-fatty acid receptor-4 (FFA4) in human cancers and cancer cell lines. Biochem Pharmacol [Internet]. 2018;150(December 2017):170–80. Available from: https://doi.org/10.1016/j.bcp.2018.02.011

68. Zhang M, Qiu S. Activation of GPR120 promotes the metastasis of breast cancer through the PI3K / Akt / NF- κ B signaling pathway. Anticancer Drugs. 2018;3:260–70.

69. Kita T, Kadochi Y, Takahashi K, Fukushima K, Yamasaki E, Uemoto T, et al. Diverse effects of G-protein-coupled free fatty acid receptors on the regulation of cellular functions in lung cancer cells. Exp Cell Res [Internet]. 2016;342(2):193–9. Available from: http://dx.doi.org/10.1016/j.yexcr.2016.03.008

70. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol [Internet]. 2011 May [cited 2014 Jul 15];12(5):489–95. Available from: http://www.sciencedirect.com/science/article/pii/S1470204510702187

71. Bruggeman AR, Kamal AH, LeBlanc TW, Ma JD, Baracos VE, Roeland EJ. Cancer Cachexia: Beyond Weight Loss. J Oncol Pract [Internet]. 2016;12(11):1163–71. Available from:

http://ascopubs.org/doi/10.1200/JOP.2016.016832

72. von Haehling S, Anker SD. Cachexia as a major underestimated and unmet medical need: Facts and numbers. J Cachexia Sarcopenia Muscle [Internet]. 2010;1(1):1–5. Available from: http://dx.doi.org/10.1016/j.ijcard.2012.09.213

73. Martin L, Sawyer M. Cancer Cachexia: Emerging pre-clinical evidence and the pathway forward to clinical trials. J Natl Cancer Inst. 2015;(2).

74. Penna F, Busquets S, Argilés JM. Experimental cancer cachexia: evolving strategies for getting closer to the human scenario. Semin Cell Dev Biol [Internet]. 2015 Sep 3 [cited 2015 Sep 8]; Available from: http://www.sciencedirect.com/science/article/pii/S1084952115001615

75. Argilés JM, Busquets S, Stemmler B, López-Soriano FJ. Cancer cachexia: understanding the molecular basis. Nat Rev Cancer [Internet]. 2014 Oct 9 [cited 2014 Oct 8];14(11):754–62. Available from: http://dx.doi.org/10.1038/nrc3829

76. Morley JE, Thomas DR, Wilson M-MG. Cachexia: pathophysiology and clinical relevance. Am J Clin Nutr [Internet]. 2006 Apr 1 [cited 2015 Aug 12];83(4):735–43. Available from: http://ajcn.nutrition.org/content/83/4/735.abstract

77. Argilés JM, López-Soriano FJ, Toledo M, Betancourt A, Serpe R, Busquets S. The cachexia score (CASCO): a new tool for staging cachectic cancer patients. J Cachexia Sarcopenia Muscle [Internet]. 2011 Jun 15 [cited 2015 Oct 1];2(2):87–93. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3117995&tool=pmcentrez&rendertype=abstract

78. Arends J, Bachmann P, Baracos V, Barthelemy N, Bertz H, Bozzetti F, et al. ESPEN guidelines on nutrition in cancer patients. Clin Nutr [Internet]. 2017;36(1):11–48. Available from: http://dx.doi.org/10.1016/j.clnu.2016.07.015

79. Arends J, Baracos V, Bertz H, Bozzetti F, Calder PC, Deutz NEP, et al. ESPEN expert group recommendations for action against cancer- related malnutrition. Clin Nutr [Internet]. 2017;36(5):1187–96. Available from: http://dx.doi.org/10.1016/j.clnu.2017.06.017

80. Benoist S, Brouquet A. Nutritional assessment and screening for malnutrition. J Visc Surg [Internet]. 2015;152(1):S3–7. Available from: http://dx.doi.org/10.1016/S1878-7886(15)30003-5

81. Stratton RJ, Hackston A, Longmore D, Dixon R, Price S, Stroud M, et al. Malnutrition in hospital outpatients and inpatients : prevalence , concurrent validity and ease of use of the ' malnutrition universal screening tool ' (' MUST ') for adults †. Br J Nutr. 2004;92:799–808.

82. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M. Guidelines for Nutrition Screening 2002. Clin Nutr. 2003;22(4):415–21.

83. Brown JC, Feliciano EMC, Caan BJ. The evolution of body composition in oncology — epidemiology , clinical trials , and the future of patient care : facts and numbers. J Cachexia Sarcopenia Muscle. 2019;1–9.

84. Mcmillan DC. The systemic inflammation-based Glasgow Prognostic Score: A decade of experience in patients with cancer. Cancer Treat Rev [Internet]. 2013;39(5):534–40. Available from: http://dx.doi.org/10.1016/j.ctrv.2012.08.003

85. Derman BA, Macklis JN, Azeem MS, Sayidine S, Basu S, Batus M, et al. Relationships between longitudinal neutrophil to lymphocyte ratios , body weight changes , and overall survival in patients with non-small cell lung cancer. BMC Cancer. 2017;17(141):1–6.

86. Loss CW, Martin L, Senesse P, Gioulbasanis I, Antoun S, Bozzetti F, et al. Diagnostic Criteria for the Classification of Cancer-Associated Weight Loss. J Clin

Oncol. 2019;33(1).

87. Kilgour RD, Vigano A, Trutschnigg B, Lucar E, Borod M, Morais JA. Handgrip strength predicts survival and is associated with markers of clinical and functional outcomes in advanced cancer patients. Support Care Cancer [Internet]. 2013;21(12):3261–70. Available from: http://link.springer.com/10.1007/s00520-013-1894-4

88. Madeddu C, Mantovani G, Gramignano G, Macciò A. Expert Opinion on Pharmacotherapy Advances in pharmacologic strategies for cancer cachexia Advances in pharmacologic strategies for cancer cachexia. Expert Opin Pharmacother Expert Opin Pharmacother [Internet]. 2015;1614(1614). Available from: http://www.tandfonline.com/action/journalInformation?journalCode=ieop20

89. Grundmann O, Yoon SL, Williams JJ. The value of bioelectrical impedance analysis and phase angle in the evaluation of malnutrition and quality of life in cancer patients—a comprehensive review. Eur J Clin Nutr. 2015;

90. Fearon K, Arends J, Baracos V. Understanding the mechanisms and treatment options in cancer cachexia. Nat Rev Clin Oncol [Internet]. 2012;10(2):90–9. Available from: http://www.nature.com/doifinder/10.1038/nrclinonc.2012.209

91. Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated cachexia. Nat Rev Dis Prim [Internet]. 2018;4:1–18. Available from: http://dx.doi.org/10.1038/nrdp.2017.105

92. Norren K Van, Dwarkasing JT, Witkamp RF. The role of hypothalamic inflammation , the hypothalamic – pituitary – adrenal axis and serotonin in the cancer anorexia – cachexia syndrome. Curr Opin Clin Nutr Metab Care. 2017;20(5):396–401.

93. Ezeoke CC, Morley JE. Pathophysiology of anorexia in the cancer cachexia syndrome. J Cachexia Sarcopenia Muscle. 2015;6(4):287–302.

94. Laviano A, Koverech A, Seelaender M. Assessing pathophysiology of cancer anorexia. Curr Opin Clin Nutr Metab Care. 2017;20(5):340–5.

95. Molfino A, Iannace A, Colaiacomo MC, Farcomeni A, Emiliani A, Gualdi G, et al. Cancer anorexia: hypothalamic activity and its association with in fl ammation and appetite-regulating peptides in lung cancer. J Cachexia Sarcopenia Muscle. 2017;8(October 2016):40–7.

96. DeBoer MD. Update on melanocortin interventions for cachexia: progress toward clinical application. Nutrition [Internet]. 2010 Feb [cited 2015 Sep 2];26(2):146–51. Available from: http://www.sciencedirect.com/science/article/pii/S0899900709002950

97. Tisdale MJ. Mechanisms of cancer cachexia. Physiol Rev [Internet]. 2009 Apr [cited 2015 Aug 23];89(2):381–410. Available from: http://physrev.physiology.org/content/89/2/381.abstract

98. Ramos EJB, Suzuki S, Marks D, Inui A, Asakawa A, Meguid MM. Cancer anorexia-cachexia syndrome: cytokines and neuropeptides. Curr Opin Clin Nutr Metab Care. 2004;7:427–34.

99. Mountjoy KG. Pro-Opiomelanocortin (POMC) Neurones, POMC-Derived Peptides, Melanocortin Receptors and Obesity: How Understanding of this System has Changed Over the Last Decade. J Neuroendocrinol. 2015;27:406–18.

100. Crujeiras AB, Carreira MC, Cabia B, Andrade S, Amil M, Casanueva FF. Leptin resistance in obesity: An epigenetic landscape. Life Sci [Internet]. 2015;140:57–63. Available from: http://dx.doi.org/10.1016/j.lfs.2015.05.003

101. Cui H, López M, Rahmouni K. The cellular and molecular bases of leptin and

ghrelin resistance in obesity. Nat Rev Endocrinol [Internet]. 2017;13(6):338–51. Available from: http://dx.doi.org/10.1038/nrendo.2016.222

102. Begenik H, Aslan M, Dulger AC, Emre H, Kemik A, Kemik O, et al. Serum leptin levels in gastric cancer patients and the relationship with insulin resistance. Arch Med Sci [Internet]. 2015;2:346–52. Available from: http://www.termedia.pl/doi/10.5114/aoms.2015.50967

103. Mondello P, Lacquaniti A, Mondello S, Bolignano D, Pitini V, Aloisi C, et al. Emerging markers of cachexia predict survival in cancer patients. BMC Cancer [Internet]. 2014;14(1):828. Available from: http://www.biomedcentral.com/1471-2407/14/828

104. Tong X, Ma Y, Zhou Q, He J, Peng B, Liu S, et al. Serum and tissue leptin in lung cancer: A meta-analysis. Oncotarget. 2017;8(12):19699–711.

105. Massadi O Al, López M, Tschöp M, Diéguez C, Nogueiras R. Current Understanding of the Hypothalamic Ghrelin Pathways Inducing Appetite and Adiposity. Trends Neurosci [Internet]. 2017;40(3):167–80. Available from: http://dx.doi.org/10.1016/j.tins.2016.12.003

106. Baldini G, Phelan KD. The melanocortin pathway and control of appetite-progress and therapeutic implications. J Endocrinol. 2019;241(1):1–33.

107. Kim JD, Leyva S, Diano S. Hormonal regulation of the hypothalamic melanocortin system. Front Physiol [Internet]. 2014 Jan 9 [cited 2015 Nov 24];5:480. Available from: http://journal.frontiersin.org/article/10.3389/fphys.2014.00480/abstract

108. Colldén G, Tschöp MH, Müller TD. Therapeutic Potential of Targeting the Ghrelin Pathway. Int J Mol Sci. 2017;18(798):1–29.

109. Shimizu Y, Nagaya N, Isobe T, Imazu M, Okumura H, Hosoda H, et al. Increased plasma ghrelin level in lung cancer cachexia. Clin Cancer Res. 2003;9(2):774–8.

110. Neary NM, Small CJ, Wren AM, Lee JL, Druce MR, Palmieri C, et al. Ghrelin Increases Energy Intake in Cancer Patients with Impaired Appetite : Acute , Randomized , Placebo-. J Clin Endocrinol Metab. 2004;89(6):2832–6.

111. Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-jones Z, et al. Serotonin Reciprocally Regulates Melanocortin Neurons to Modulate Food Intake. Neuron. 2006;51:239–49.

112. Dwarkasing JT, Marks DL, Witkamp RF, Norren K Van. Hypothalamic inflammation and food intake regulation during chronic illness. Peptides [Internet]. 2016;77:60–6. Available from: http://dx.doi.org/10.1016/j.peptides.2015.06.011

113. Amitani M, Asakawa A, Amitani H, Inui A. Control of food intake and muscle wasting in cachexia. Int J Biochem Cell Biol [Internet]. 2013;45(10):2179–85. Available from: http://dx.doi.org/10.1016/j.biocel.2013.07.016

114. Makarenko IG, Meguid MM, Gatto L, Goncalves CG, Jb E, Chen C, et al. Hypothalamic 5-HT 1B -receptor changes in anorectic tumor bearing rats. Neurosci Lett. 2005;376:71–5.

115. Makarenko IG, Meguid MM, Gatto L, Chen C, Ramos EJB, Goncalves CG, et al. Normalization of hypothalamic serotonin (5-HT 1B) receptor and NPY in cancer anorexia after tumor resection : An immunocytochemical study. Neurosci Lett. 2005;383:322–7.

116. Molfino A, Gioia G, Fanelli FR, Laviano A. Contribution of Neuroinflammation to the Pathogenesis of Cancer Cachexia. Mediators Inflamm. 2015;

117. Sánchez-Lara K, Arrieta O, Pasaye E, Laviano A, Mercadillo R, Sosa-Sánchez R, et al. Brain activity correlated with food preferences : A functional study comparing advanced non-small cell lung cancer patients with and without anorexia. Nutrition. 2013;29:1013–9.

118. Arruda AP, Milanski M, Romanatto T, Solon C, Coope A, Alberici LC, et al. Hypothalamic Actions of Tumor Necrosis Factor Provide the Thermogenic Core for the Wastage Syndrome in Cachexia. Endocrinology. 2010;151(February):683–94.

119. Csetényi B, Hormay E, Szabó I, Takács G, Nagy B, László K, et al. Food and water intake , body temperature and metabolic consequences of interleukin-1 β microinjection into the cingulate cortex of the rat. Behav Brain Res [Internet]. 2017;331(March):115–22. Available from: https://doi.org/10.1016/j.bbr.2017.05.041

120. Paulsen Ø, Laird B, Aass N, Lea T, Fayers P, Kaasa S, et al. The relationship between pro-inflammatory cytokines and pain , appetite and fatigue in patients with advanced cancer. PLoS One. 2017;12:1–16.

121. Burfeind KG, Michaelis KA, Marks DL, Health O, Health O. The central role of hypothalamic inflammation in the acute ilness response and cachexia. Semin Cell Dev Biol. 2016;54:42–52.

122. Whitaker KW, Reyes TM. Central blockade of melanocortin receptors attenuates the metabolic and locomotor responses to peripheral interleukin-1b administration. Neuropharmacology. 2008;54:509–20.

123. Romanatto T, Cesquini M, Amaral ME, Roman ÉA, Moraes JC, Torsoni MA, et al. TNF- a acts in the hypothalamus inhibiting food intake and increasing the respiratory quotient — Effects on leptin and insulin signaling pathways. Peptides. 2007;28:1050–8.

124. Biology C, Johns N, Stephens NA, Fearon KCH. The International Journal of Biochemistry Muscle wasting in cancer ☪. Int J Biochem Cell Biol [Internet]. 2013;45(10):2215–29. Available from:

http://dx.doi.org/10.1016/j.biocel.2013.05.032

125. Sandri M. Protein Breakdown in Cancer Cachexia. Semin Cell Dev Biol [Internet]. 2015; Available from: http://linkinghub.elsevier.com/retrieve/pii/S1084952115002487

126. Cohen S, Nathan JA, Goldberg AL. Muscle wasting in disease: molecular mechanisms and promising therapies. Nat Rev Drug Discov [Internet]. 2015 Jan [cited 2015 Aug 6];14(1):58–74. Available from: http://dx.doi.org/10.1038/nrd4467

127. Bodine SC, Baehr LM. Skeletal muscle atrophy and the E3 ubiquitin ligases MuRF1 and MAFbx/atrogin-1. AJP Endocrinol Metab [Internet]. 2014;307(6):E469–84. Available from: http://ajpendo.physiology.org/cgi/doi/10.1152/ajpendo.00204.2014

128. Mckinnell IW, Rudnicki MA. Molecular Mechanisms of Muscle Atrophy. 2004;119:907–10.

129. Banerjee A, Guttridge DC. Mechanisms for maintaining muscle. Curr Opin Support Palliat Care [Internet]. 2012;6(4):451–6. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23108340

130. Agustsson T, D'souza M a, Nowak G, Isaksson B. Mechanisms for skeletal muscle insulin resistance in patients with pancreatic ductal adenocarcinoma. Nutrition [Internet]. 2011;27(7–8):796–801. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21050717

131. Asp ML, Tian M, Wendel AA, Belury MA. Evidence for the contribution of insulin resistance to the development of cachexia in tumor-bearing mice. Int J Cancer [Internet]. 2010;126(3):756–63. Available from: papers2://publication/uuid/56287966-5D96-40AD-B1CE-

D9141EFB22FD%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/19634137

132. Landskron G, Fuente M De, Thuwajit P, Thuwajit C, Hermoso MA. Chronic Inflammation and Cytokines in the Tumor Microenvironment. 2014;2014.

133. Fearon KCH, Glass DJ, Guttridge DC. Cancer Cachexia: Mediators, Signaling,and Metabolic Pathways. Cell Metab. 2012;16:153–66.

134. Mendes MCS, Pimentel GD, Costa FO, Carvalheira JBC. Molecular and neuroendocrine mechanisms of cancer cachexia. J Endocrinol [Internet]. 2015;226(3):R29–43. Available from: http://joe.endocrinology-journals.org/lookup/doi/10.1530/JOE-15-0170

135. Lu JJ, Wang Q, Xie LH, Zhang Q, Sun SH. Tumor necrosis factor-like weak inducer of apoptosis regulates quadriceps muscle atrophy and fiber-type alteration in a rat model of chronic obstructive pulmonary disease. Tob Induc Dis. 2017;15(1):1–10.

136. Bowerman M, Salsac C, Coque E, Eiselt É, Deschaumes RG, Brodovitch A, et al. Tweak regulates astrogliosis, microgliosis and skeletal muscle atrophy in a mouse model of amyotrophic lateral sclerosis. Hum Mol Genet. 2015;24(12):3440–56.

137. Zhou J, Liu B, Liang C, Li Y, Song YH. Cytokine Signaling in Skeletal Muscle Wasting. Trends Endocrinol Metab [Internet]. 2016;27(5):335–47. Available from: http://dx.doi.org/10.1016/j.tem.2016.03.002

138. Lee S. Genetic Analysis of the Role of Proteolysis in the Activation of Latent Myostatin. PLoS One. 2008;3(2).

139. Han HQ, Mitch WE. Targeting the myostatin signaling pathway to treat muscle wasting diseases. Curr Opin Support Palliat Care. 2011;5:334–41.

140. Waning DL, Mohammad KS, Reiken S, Xie W, Andersson DC, John S, et al. Excess TGF-β mediates muscle weakness associated with bone metastases in mice.

Nat Med [Internet]. 2015;21(11):1–14. Available from: http://www.nature.com/doifinder/10.1038/nm.3961

141. Ebadi M, Mazurak V. Evidence and Mechanisms of Fat Depletion in Cancer. Nutrients [Internet]. 2014;6(11):5280–97. Available from: http://www.mdpi.com/2072-6643/6/11/5280/

142. Bing C, Trayhurn P. Regulation of adipose tissue metabolism in cancer cachexia. Curr Opin Clin Nutr Metab Care. 2008;11:201–7.

143. Wang F, Liu H, Hu L, Liu Y, Duan Y, Cui R, et al. The Warburg effect in human pancreatic cancer cells triggers cachexia in athymic mice carrying the cancer cells. BMC Cancer. 2018;18(6):1–12.

144. Tisdale MJ. Cachexia in Cancer Patients. Nat Rev Cancer. 2002;2:862–71.

145. Patel HJ, Patel BM. TNF-α and cancer cachexia: Molecular insights and clinical implications. Life Sciences. 2017.

146. Han J, Meng Q, Shen L, Wu G. Interleukin-6 induces fat loss in cancer cachexia by promoting white adipose tissue lipolysis and browning. Lipids Health Dis. 2018;17(1):1–8.

147. Kir S, White JP, Kleiner S, Kazak L, Cohen P, Baracos VE, et al. Tumour-derived PTH-related protein triggers adipose tissue browning and cancer cachexia. Nature [Internet]. 2014;513(7516):100–4. Available from: http://dx.doi.org/10.1038/nature13528

148. Ebadi M, Mazurak VC. Potential Biomarkers of Fat Loss as a Feature of Cancer Cachexia. 2015;2015.

149. Arora GK, Iyengar P, Infante RE, Arora GK, Gupta A, Narayanan S, et al. Cachexia-associated adipose loss induced by tumor-secreted leukemia inhibitory factor is counterbalanced by decreased leptin Graphical abstract Find the latest

version : Cachexia-associated adipose loss induced by tumor-secreted leukemia inhibitory factor. J Clin Investig Insight. 2018;3(14).

150. Bolsoni-Lopes A, Alonso-Vale MI. Lipolysis and lipases in white adipose tissue - An update. Arch Endocrinol Metab. 2015;59(4):335–42.

151. Evans B, Merlin J, Bengtsson T, Hutchinson D. Adrenoceptors in white, brown and brite adipocytes. Br J Pharmacol. 2019;

152. Montanari T, Poscic N, Colitti M. Factors involved in white-to-brown adipose tissue conversion and in thermogenesis : a review. Obes Rev. 2017;(May):495–513.

153. Dempersmier J, Sul HS. Shades of brown: a model for thermogenic fat. Front Endocrinol (Lausanne). 2015;6(May):1–6.

154. Ruiz Garcia V, López-Briz E, Carbonell Sanchis R, Gonzaøvez Perales J, Bort-Marti S. Megestrol acetate for treatment of anorexia-cachexia syndrome . PubMed Commons. 2013;(3):7–8.

155. Yeh SS, Schuster MW. Megestrol acetate in cachexia and anorexia. Int J Nanomedicine. 2006;1(4):411–6.

156. Katakami N, Uchino J, Yokoyama T, Naito T, Kondo M, Yamada K, et al. Anamorelin (ONO-7643) for the treatment of patients with non–small cell lung cancer and cachexia: Results from a randomized, double-blind, placebo-controlled, multicenter study of Japanese patients (ONO-7643-04). Cancer. 2018;124(3):606–16.

157. Prommer E. Oncology Update: Anamorelin. Palliat Care. 2017;10.

158. Nishie K, Yamamoto S, Nagata C, Koizumi T, Hanaoka M. Anamorelin for advanced non-small-cell lung cancer with cachexia: Systematic review and meta-analysis. Lung Cancer [Internet]. 2017;112(April):25–34. Available from:

http://dx.doi.org/10.1016/j.lungcan.2017.07.023

159. Blauwhoff-Buskermolen S, Langius JAE, Heijboer AC, Becker A, de van der Schueren MAE, Verheul HMW. Plasma ghrelin levels are associated with anorexia but not cachexia in patients with NSCLC. Front Physiol. 2017;8(MAR):1–5.

160. Chen J, Splenser A, Guillory B, Luo J, Mendiratta M, Belinova B, et al. Ghrelin prevents tumour- and cisplatin-induced muscle wasting: characterization of multiple mechanisms involved. J Cachexia Sarcopenia Muscle [Internet]. 2015;(April):n/a-n/a. Available from: http://doi.wiley.com/10.1002/jcsm.12023

161. Conte E, Camerino GM, Mele A, De Bellis M, Pierno S, Rana F, et al. Growth hormone secretagogues prevent dysregulation of skeletal muscle calcium homeostasis in a rat model of cisplatin-induced cachexia. J Cachexia Sarcopenia Muscle. 2017;8(3):386–404.

162. Ropelle ER, Pauli JR, Zecchin KG, Ueno M, de Souza CT, Morari J, et al. A Central Role for Neuronal Amp Activated Protein Kinase (Ampk) in Cancer-Induced Anorexia. Endocrinology [Internet]. 2007;148(November):5220–9. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=17717055

163. Moreira VM, Franco CC da S, Prates KV, Gomes RM, de Moraes AMP, Ribeiro TA, et al. Aerobic exercise training attenuates tumor growth and reduces insulin secretion in walker 256 tumor-bearing rats. Front Physiol. 2018;9(MAY):1–14.

164. Solheim TS, Laird BJA, Balstad TR, Bye A, Stene G, Baracos V, et al. Cancer cachexia: rationale for the MENAC (Multimodal-Exercise, Nutrition and Anti-inflammatory medication for Cachexia) trial. BMJ Support Palliat Care [Internet]. 2018;bmjspcare-2017-001440. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/29440149

165. Aoyagi T, Terracina KP, Raza A, Matsubara H, Takabe K. Cancer cachexia, mechanism and treatment. World J Gastrointest Oncol [Internet]. 2015;7(4):17–29. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4398892&tool=pmcentrez&rendertype=abstract

166. Pappalardo G, Almeida A, Ravasco P. Eicosapentaenoic acid in cancer improves body composition and modulates metabolism. Nutrition [Internet]. 2015 Apr [cited 2015 Aug 24];31(4):549–55. Available from: http://www.sciencedirect.com/science/article/pii/S0899900714005012

167. Murphy R a, Yeung E, Mazurak VC, Mourtzakis M. Influence of eicosapentaenoic acid supplementation on lean body mass in cancer cachexia. Br J Cancer [Internet]. 2011;105(10):1469–73. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3242518&tool=pmcentrez&rendertype=abstract

168. Nabavi SF, Bilotto S, Russo GL, Orhan IE, Habtemariam S, Daglia M, et al. Omega-3 polyunsaturated fatty acids and cancer: lessons learned from clinical trials. Cancer Metastasis Rev [Internet]. 2015;34(3):359–80. Available from: http://link.springer.com/10.1007/s10555-015-9572-2

169. Lavriv DS, Neves PM, Ravasco P. Should omega-3 fatty acids be used for adjuvant treatment of cancer cachexia? Clin Nutr ESPEN [Internet]. 2018;25:18–25. Available from: https://doi.org/10.1016/j.clnesp.2018.02.006

170. WHO. Cancer fact sheet [Internet]. 2019 [cited 2019 Jun 10]. Available from: https://www.who.int/en/news-room/fact-sheets/detail/cancer

171. Argilés JM, Stemmler B, López-Soriano FJ, Busquets S. Inter-tissue

communication in cancer cachexia. Nat Rev Endocrinol [Internet]. 2018; Available from: http://dx.doi.org/10.1038/s41574-018-0123-0

172. Von Haehling S, Anker SD. Cachexia as major underestimated unmet medical need: Facts and numbers. Int J Cardiol. 2012;161:121–3.

173. Seelaender M, Laviano A, Busquets S, Püschel GP, Margaria T, Jr MLB. Inflammation in Cachexia. 2015;2015:2–4.

174. Mirza KA, Tisdale MJ. Role of Ca 2 + in proteolysis-inducing factor (PIF) - induced atrophy of skeletal muscle. Cell Signal [Internet]. 2012;24(11):2118–22. Available from: http://dx.doi.org/10.1016/j.cellsig.2012.07.016

175. Tsoli M, Swarbrick MM, Robertson GR. Lipolytic and thermogenic depletion of adipose tissue in cancer cachexia. Semin Cell Dev Biol [Internet]. 2015;1–14. Available from: http://linkinghub.elsevier.com/retrieve/pii/S1084952115002463

176. Simopoulos AP. An Increase in the Omega-6/Omega-3 Fatty Acid Ratio Increases the Risk for Obesity. Nutrients. 2016;8:1–17.

177. Serhan CN, Chiang N, Dalli J, Levy BD. Lipid Mediators in the Resolution of Inflammation. Cold Spring Harb Perspect Biol. 2015;7.

178. Mancini AD, Poitout V. The fatty acid receptor FFA1/GPR40 a decade later: How much do we know? Trends Endocrinol Metab [Internet]. 2013;24(8):398–407. Available from: http://dx.doi.org/10.1016/j.tem.2013.03.003

179. Nishinaka T, Yamashita T, Nakamoto K, Kasuya F, Tokuyama S. Involvement of the Long-Chain Fatty Acid Receptor GPR40 in Depression-Related Behavior. J Pharmacol Sci. 2014;115:112–5.

180. Khan MZ, Zhuang X, He L. GPR40 receptor activation leads to CREB phosphorylation and improves cognitive performance in an Alzheimer's disease mouse model. Neurobiol Learn Mem [Internet]. 2016;131:46–55. Available from:

http://dx.doi.org/10.1016/j.nlm.2016.03.006

181. Oliveira V, Marinho R, Vitorino D, Santos GA, Moraes JC, Dragano N, et al. Diets containing α-linolenic (ω3) or oleic (ω9) fatty acids rescues obese mice from insulin resistance. Endocrinology. 2015;156(11):4033–46.

182. Dátilo MN, Ramos M, Formigari G, Rodrigues P, de Moura L, da Silva A, et al. Omega-3 from Flaxseed Oil Protects Obese Mice Against Diabetic Retinopathy Through GPR120 Receptor. Sci Rep. 2018;8(September):1–13.

183. Moura-Assis A, Afonso MS, de Oliveira V, Morari J, dos Santos GA, Koike M, et al. Flaxseed oil rich in omega-3 protects aorta against inflammation and endoplasmic reticulum stress partially mediated by GPR120 receptor in obese, diabetic and dyslipidemic mice models. J Nutr Biochem [Internet]. 2018;53:9–19. Available from: https://doi.org/10.1016/j.jnutbio.2017.09.015

184. Gaspar R, Veiga C, Bessi M, Dátilo M, Sant'Ana M, Rodrigues P, et al. Unsaturated fatty acids from flaxseed oil and exercise modulate GPR120 but not GPR40 in the liver of obese mice : a new anti-inflammatory approach. J Nutr Biochem [Internet]. 2019;66:52–62. Available from: https://doi.org/10.1016/j.jnutbio.2018.12.003

185. Ma D, Tao B, Warashina S, Kotani S, Lu L, Kaplamadzhiev DB, et al. Expression of free fatty acid receptor GPR40 in the central nervous system of adult monkeys. Neurosci Res [Internet]. 2007 Aug [cited 2014 Jan 31];58(4):394–401. Available from: http://www.sciencedirect.com/science/article/pii/S0168010207001563

186. Hopkins MM, Meier KE. Free Fatty Acid Receptors and Cancer : From Nutrition to Pharmacology. Handb Exp Pharmacol. 2016;

187. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG. Improving Bioscience Research Reporting : The ARRIVE Guidelines for Reporting Animal

Research. Plos Biol. 2010;8(6):6–10.

188. Hudson BD, Shimpukade B, Mackenzie AE, Butcher AJ, Pediani JD, Christiansen E, et al. The pharmacology of TUG-891, a potent and selective agonist of the free fatty acid receptor 4 (FFA4/GPR120), demonstrates both potential opportunity and possible challenges to therapeutic agonism. Mol Pharmacol [Internet]. 2013;84(5):710–25. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3807074&tool=pmcentrez&rendertype=abstract

189. Sparks SM, Chen G, Collins JL, Danger D, Dock ST, Jayawickreme C, et al. Identification of diarylsulfonamides as agonists of the free fatty acid receptor 4 (FFA4/GPR120). Bioorganic Med Chem Lett [Internet]. 2014;24(14):3100–3. Available from: http://dx.doi.org/10.1016/j.bmcl.2014.05.012

190. Schiessel DL, Yamazaki RK, Kryczyk M, Coelho I, Yamaguchi AA, Pequito DCT, et al. α-Linolenic Fatty Acid Supplementation Decreases Tumor Growth and Cachexia Parameters in Walker 256 Tumor-Bearing Rats. Nutr Cancer. 2015;67(5):839–46.

191. Gong Z, Yoshimura M, Aizawa S, Kurotani R, Zigman JM, Sakai T, et al. G protein-coupled receptor 120 signaling regulates ghrelin secretion in vivo and in vitro. Am J Physiol Endocrinol Metab. 2014;12:28–35.

192. Silva RBM, Greggio S, Venturin GT, da Costa JC, Gomez MV, Campos MM. Beneficial Effects of the Calcium Channel Blocker CTK 01512-2 in a Mouse Model of Multiple Sclerosis. Mol Neurobiol. 2018;

193. Dagnino AP, Silva RBM, Chagastelles PC, Pereira TCB, Venturin GT, Greggio S, et al. Nociceptin/orphanin FQ receptor modulates painful and fatigue symptoms in a mouse model of fibromyalgia. Pain. 2019;

194. Bertelli JA, Mira JC. The grasping test: a simple behavioral method for objective quantitative assessment of peripheral nerve regeneration in the rat. J Neurosci Methods. 1995;58(1–2):151–5.

195. Amaral MEA, Nery LR, Leite CE, Filgueira W, Junior DA, Campos MM. Pre-clinical effects of metformin and aspirin on the cell lines of different breast cancer subtypes. Invest New Drugs. 2018;36:782–96.

196. Penet MF, Gadiya MM, Krishnamachary B, Nimmagadda S, Pomper MG, Artemov D, et al. Metabolic signatures imaged in cancer-induced cachexia. Cancer Res. 2011;71(22):6948–56.

197. Bellaver B, Rocha AS, Souza DG, Leffa DT, De Bastiani MA, Schu G, et al. Activated peripheral blood mononuclear cell mediators trigger astrocyte reactivity. Brain Behav Immun. 2019;(May).

198. Su X, Cheng K, Jeon J, Shen B, Venturin GT, Hu X, et al. Comparison of Two Site-Specifically 18F-Labeled Affibodies for PET Imaging of EGFR Positive Tumors. Mol Pharm. 2014;11:3947–56.

199. Harada S, Haruna Y, Aizawa F, Matsuura W, Nakamoto K. Involvement of GPR40 , a long-chain free fatty acid receptor , in the production of central post-stroke pain after global cerebral ischemia. Eur J Pharmacol [Internet]. 2014;744:115–23. Available from: http://dx.doi.org/10.1016/j.ejphar.2014.09.036

200. Dupré A, Malik HZ. Inflammation and cancer: What a surgical oncologist should know. Eur J Surg Oncol. 2018;44(5):566–70.

201. Hanai N, Terada H, Hirakawa H, Suzuki H, Nishikawa D, Beppu S, et al. Prospective randomized investigation implementing immunonutritional therapy using a nutritional supplement with a high blend ratio of ω-3 fatty acids during the perioperative period for head and neck carcinomas. Jpn J Clin Oncol.

2018;48(4):356–61.

202. Abe K, Uwagawa T, Haruki K, Takano Y. Effects of ω-3 Fatty Acid Supplementation in Patients with Bile Duct or Pancreatic Cancer Undergoing Chemotherapy. Anticancer Res. 2018;2375:2369–75.

203. Henriques F, Lope MA, Fr FO, Pamela K. Toll-Like Receptor-4 Disruption Suppresses Adipose Tissue Remodeling and Increases Survival in Cancer Cachexia Syndrome. Sci Rep. 2018;8(November):1–14.

204. Chen X, Wu Y, Yang T, Wei M, Wang Y, Deng X, et al. Salidroside alleviates cachexia symptoms in mouse models of cancer cachexia via activating mTOR signalling. J Cachexia Sarcopenia Muscle. 2016;7(January):224–32.

205. Carnier M, Silva FP, Miranda DA De, Hachul CL, Rischiteli A, Neto NIP, et al. Diet Supplemented with Chia Flour did not Modified the Inflammatory Process and Tumor Development in Wistar Rats Inoculated Walker 256 Cells. Nutr Cancer [Internet]. 2018;0(0):1–10. Available from: https://doi.org/10.1080/01635581.2018.1502329

206. Hajjaji N, Couet C, Besson P, Bougnoux P. DHA Effect on Chemotherapy-Induced Body Weight Loss : An Exploratory Study in a Rodent Model of Mammary Tumors. Nutr Cancer. 2012;61(7):37–41.

207. Deval C, Capel F, Laillet B, Polge C, Béchet D, Taillandier D, et al. Docosahexaenoic acid-supplementation prior to fasting prevents muscle atrophy in mice. J Cachexia Sarcopenia Muscle. 2016;7(5):587–603.

208. Gorjao R, Miguel C, Duarte T, Serdan A, Leonardo V, Diniz S, et al. New insights on the regulation of cancer cachexia by N-3 polyunsaturated fatty acids. Pharmacol Ther [Internet]. 2018;(xxxx). Available from: https://doi.org/10.1016/j.pharmthera.2018.12.001

209. Chu X, Zhou Q, Xu Y, Jiang J, Li Q, Zhou Q, et al. Aberrant fatty acid profile and FFAR4 signaling confer endocrine resistance in breast cancer. J Exp Clin Cancer Res. 2019;38(1):1–18.

210. Zhao Y, Zhang L, Yan A, Chen D, Xie R, Liu Y, et al. Grifolic acid induces GH3 adenoma cell death by inhibiting ATP production through a GPR120-independent mechanism. BMC Pharmacol Toxicol. 2018;19(1):1–9.

211. Hopkins MM, Meier KE. Free fatty acid receptor (FFAR) agonists inhibit proliferation of human ovarian cancer cells. Prostaglandins Leukot Essent Fat Acids [Internet]. 2017;122(December 2016):24–9. Available from: http://dx.doi.org/10.1016/j.plefa.2017.06.013

212. Kandarian SC, Nosacka RL, Delitto AE, Judge AR, Judge SM, Ganey JD, et al. Tumour-derived leukaemia inhibitory factor is a major driver of cancer cachexia and morbidity in C26 tumour-bearing mice. 2018;(September):1109–20.

213. Gotoh C, Hong Y, Iga T, Hishikawa D, Suzuki Y, Song S, et al. The regulation of adipogenesis through GPR120. Biochem Biophys Res Commun. 2007;354:591–7.

214. Mckenzie C V, Colonne CK, Fraser ST. Splenomegaly : Pathophysiological bases and therapeutic options. Int J Biochem Cell Biol. 2018;94:40–3.

215. Toledo M, Penna F, Busquets S, López-Soriano FJ, Argilés JM. Distinct behaviour of sorafenib in experimental cachexia-inducing tumours: the role of STAT3. PLoS One [Internet]. 2014;9(12):e113931. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4250056&tool=pmcentrez&rendertype=abstract

216. Zhang X, Zhang F, Qiao W, Zhang X, Zhao Z, Li M. Low Hematocrit Is a Strong Predictor of Poor Prognosis in Lung Cancer Patients. Biomed Res Int. 2018;

217. Kooshki A, Taleban FA, Tabibi H, Hedayati M, Kooshki A, Taleban FA, et al.

Effects of Omega-3 Fatty Acids on Serum Lipids , Lipoprotein (a), and Hematologic Factors in Hemodialysis Patients Effects of Omega-3 Fatty Acids on Serum Lipids , Lipoprotein (a), and Hematologic Factors in Hemodialysis Patients. Ren Fail. 2011;6049.

218. Huang TH, Chiu YH, Chan YL, Chiu YH, Wang H, Huang KC, et al. Prophylactic administration of fucoidan represses cancer metastasis by inhibiting vascular endothelial growth factor (VEGF) and matrix metalloproteinases (MMPs) in Lewis tumor-bearing mice. Mar Drugs. 2015;13(4):1882–900.

219. Khadge S, Thiele GM, Graham J, Timothy S, Lynell RM, Black PN, et al. Long-chain omega-3 polyunsaturated fatty acids decrease mammary tumor growth , multiorgan metastasis and enhance survival. Clin Exp Metastasis [Internet]. 2018;35(8):797–818. Available from: http://dx.doi.org/10.1007/s10585-018-9941-7

220. Shirai Y, Okugawa Y, Hishida A, Ogawa A, Okamoto K. Fish oil-enriched nutrition combined with systemic chemotherapy for gastrointestinal cancer patients with cancer cachexia. Sci Rep. 2017;(November 2016):1–9.

221. Baker LA, Martin NRW, Kimber MC, Lewis MP. Resolvin E1 (RvE1) attenuates LPS induced inflammation and subsequent atrophy in C2C12 myotubes. J Cell Biochem. 2018;(February):6094–103.

222. Brown JL, Lee DE, Rosa-caldwell ME, Brown LA, Perry RA, Haynie WS, et al. Protein imbalance in the development of skeletal muscle wasting in tumour-bearing mice. J Cachexia Sarcopenia Muscle. 2018;(October):987–1002.

223. Folker ES, Baylies MK. Nuclear positioning in muscle development and disease. Front Physiol. 2013;4(December):1–10.

224. Judge SM, Nosacka RL, Delitto D, Gerber MH, Cameron ME, Trevino JG, et al.

Skeletal Muscle Fibrosis in Pancreatic Cancer Patients with Respect to Survival. JNCI Cancer Spectr. 2019;2(3):1–13.

225. Inaba S, Hinohara A, Id MT, Tsujikawa K. Muscle regeneration is disrupted by cancer cachexia without loss of muscle stem cell potential. PLoS One. 2018;1–15.

226. Chevalier S, Farsijani S. Cancer cachexia and diabetes: similarities in metabolic alterations and possible treatment(.). Appl Physiol Nutr Metab. 2014;

227. Watterson KR, Hudson BD, Ulven T, Milligan G. Treatment of type 2 diabetes by free Fatty Acid receptor agonists. Front Endocrinol (Lausanne) [Internet]. 2014 Jan 28 [cited 2014 Nov 5];5:137. Available from: http://journal.frontiersin.org/Journal/10.3389/fendo.2014.00137/abstract

228. Holliday ND, Watson S, Brown AJH, Smith NJ, Cardiac VC. Drug discovery opportunities and challenges at G protein coupled receptors for long chain free fatty acids. Front Endocrinol (Lausanne). 2012;2(January):1–12.

229. Satapati S, Qian Y, Wu MS, Petrov A, Dai G, Wang S, et al. GPR120 suppresses adipose tissue lipolysis and synergizes with GPR40 in antidiabetic efficacy. J Lipid Res. 2017;58.

230. Das SK, Eder S, Schauer S, Diwoky C, Temmel H, Guertl B, et al. Adipose Triglyceride Lipase Contributes to Cancer-Associated Cachexia. Science (80-). 2013;233(2011).

231. Mann A, Thompson A, Robbins N, Blomkalns AL. Localization , Identification , and Excision of Murine Adipose Depots. J Vis Exp. 2014;(December):1–7.

232. Michaelis KA, Zhu X, Burfeind KG, Krasnow SM, Levasseur PR, Morgan TK, et al. Establishment and characterization of a novel murine model of pancreatic cancer cachexia. J Cachexia Sarcopenia Muscle. 2017;8(July):824–38.

233. Parmar MP, Vanderbyl BL, Kanbalian M, Windholz TY, Tran A, Jagoe RT. A

multidisciplinary rehabilitation programme for cancer cachexia improves quality of life. BMJ Support Palliat Care. 2017;7:441–9.

234. Wallengren O, Lundholm K, Bosaeus I. Diagnostic criteria of cancer cachexia : relation to quality of life , exercise capacity , and survival in unselected palliative care patients. Support Care Cancer. 2013;21:1569–77.

235. Campos CA, Bowen AJ, Han S, Wisse BE, Palmiter RD, Schwartz MW. Cancer-induced anorexia and malaise are mediated by CGRP neurons in the parabrachial nucleus. Nat Neurosci. 2017;20(7):934–42.

236. Iwata Y, Suzuki N, Ohtake H, Kamauchi S, Hashimoto N, Kiyono T, et al. Cancer cachexia causes skeletal muscle damage via transient receptor potential vanilloid 2-independent mechanisms, unlike muscular dystrophy. J Cachexia Sarcopenia Muscle. 2016;(November 2015):366–76.

237. Nakamoto K, Nishinaka T, Sato N, Mankura M, Koyama Y, Kasuya F, et al. Hypothalamic GPR40 Signaling Activated by Free Long Chain Fatty Acids Suppresses CFA-Induced Inflammatory Chronic Pain. Price TJ, editor. PLoS One [Internet]. 2013 Jan [cited 2014 Jan 15];8(12):e81563. Available from: http://dx.plos.org/10.1371/journal.pone.0081563

238. Cintra D, Ropelle E, Moraes J, Pauli J, Morari J, de Souza C, et al. Unsaturated Fatty Acids Revert Diet-Induced Hypothalamic Inflammation in Obesity. PLoS One. 2012;7(1).

239. Pin F, Busquets S, Toledo M, Camperi A, Lopez-Soriano FJ, Costelli P, et al. Combination of exercise training and erythropoietin prevents cancer-induced muscle alterations. Oncotarget [Internet]. 2015;6(41):43202–15. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26636649

240. Murphy KT, Struk A, Malcontenti-Wilson C, Christophi C, Lynch GS.

Physiological characterization of a mouse model of cachexia in colorectal liver metastases. Am J Physiol Regul Integr Comp Physiol [Internet]. 2013;304(10):R854-64. Available from: http://www.ncbi.nlm.nih.gov/pubmed/23485871

241. Mauricio AF, Minatel E, Neto HS, Marques MJ. Effects of fish oil containing eicosapentaenoic acid and docosahexaenoic acid on dystrophic mdx mice. Clin Nutr [Internet]. 2013;32(4):636–42. Available from: http://dx.doi.org/10.1016/j.clnu.2012.11.013

242. Kim N, Lee JO, Lee HJ, Kim HI, Kim JK, Lee YW, et al. Endogenous ligand for GPR120, docosahexaenoic acid, exerts benign metabolic effects on the skeletal muscles via AMP-activated protein kinase pathway. J Biol Chem. 2015;290(33):20438–47.

243. Konsman JP, Blomqvist A. Forebrain patterns of c-Fos and FosB induction during cancer-associated anorexia – cachexia in rat. Eur J Neurosci. 2005;21(March):2752–66.

244. Zhang W, Ning N, Li X, Niu G, Bai L, Guo Y. Changes of Brain Glucose Metabolism in the Pretreatment Patients with Non-Small Cell Lung Cancer: A Retrospective PET / CT Study. PLoS One. 2016;1–10.

245. Khan MZ, He L. The role of polyunsaturated fatty acids and GPR40 receptor in brain. Neuropharmacology [Internet]. 2017;113(24):639–51. Available from: http://dx.doi.org/10.1016/j.neuropharm.2015.05.013

246. Moriyama R, Deura C, Imoto S, Nose K, Fukushima N. Expression of the long-chain fatty acid receptor GPR120 in the gonadotropes of the mouse anterior pituitary gland. Histochem Cell Biol. 2015;143:21–7.

247. Yin J, Li H, Meng C, Chen D, Chen Z, Wang Y, et al. Inhibitory effects of omega-

3 fatty acids on early brain injury after subarachnoid hemorrhage in rats : Possible involvement of G binding protein-1 signaling pathway. Int J Biochem Cell Biol [Internet]. 2016;75:11–22. Available from: http://dx.doi.org/10.1016/j.biocel.2016.03.008

248. Hara T, Kashihara D, Ichimura A, Kimura I, Tsujimoto G, Hirasawa A. Role of free fatty acid receptors in the regulation of energy metabolism. BBA - Mol Cell Biol Lipids [Internet]. 2014;1841(9):1292–300. Available from: http://dx.doi.org/10.1016/j.bbalip.2014.06.002

249. Oliveira V, Marinho R, Vitorino D, Santos GA, Moraes JC, Dragano N, et al. Diets containing alpha-linolenic (n-3) or oleic (n-9) fatty acids rescues obese mice from insulin resistance. Endocrinology. 2015;156(11):4033–46.

250. Philippe C, Wauquier F, Lyan B, Wittrant Y. GPR40 , a free fatty acid receptor , differentially impacts osteoblast behavior depending on differentiation stage and environment. Mol Cell Biochem. 2016;412:197–208.

251. Philippe C, Wauquier F, Leotoing L, Coxam V, Wittrant Y. GW9508, a free fatty acid receptor agonist, specifically induces cell death in bone resorbing precursor cells through increased oxidative stress from mitochondrial origin. Exp Cell Res [Internet]. 2013;319(19):3035–41. Available from: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?cmd=Retrieve&db=PubMed&dopt=Citation&list_uids=23973666

252. Kohara K, Obata A, Kimura T, Shimoda M, Moriuchi S, Okauchi S. Suppression of free fatty acid receptor 1 expression in pancreatic β-cells in obese type 2 diabetic db / db mice : a potential role of pancreatic and duodenal homeobox factor 1. Endocr J. 2019;66(1):43–50.

ANEXO A: Aprovação da Comissão de Ética no Uso de Animais - PUCRS

S I P E S Q

Sistema de Pesquisas da PUCRS

Código SIPESQ: 7164 Porto Alegre, 25 de maio de 2016.

Prezado(a) Pesquisador(a),

A Comissão de Ética no Uso de Animais da PUCRS apreciou e aprovou o Projeto de Pesquisa "O PAPEL DOS RECEPTORES GPR40 E GPR120 EM MODELO DE CAQUEXIA ASSOCIADA AO CÂNCER EM CAMUNDONGOS" coordenado por MARIA MARTHA CAMPOS.

Sua investigação, respeitando com detalhe as descrições contidas no projeto e formulários avaliados pela CEUA, está autorizada a partir da presente data.

Informamos que é necessário o encaminhamento de relatório final quando finalizar esta investigação. Adicionalmente, ressaltamos que conforme previsto na Lei no. 11.794, de 08 de outubro de 2008 (Lei Arouca), que regulamenta os procedimentos para o uso científico de animais, é função da CEUA zelar pelo cumprimento dos procedimentos informados, realizando inspeções periódicas nos locais de pesquisa.

Nº de Animais	Espécie	Duração do Projeto
480	Mus musculus	25/05/2016 - 25/05/2019

Atenciosamente,

Comissão de Ética no Uso de Animais (CEUA)

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