UNIVERSIDADE D
COIMBRA

Luana Souto Rocha

Relatórios de Estágio sob a orientação da Doutora Alexandra Gonçalves e da Dra. Amália Alves e Monografia intitulada "The relationship between Cholesterol and Alzheimer's disease" sob a orientação da Professora Doutora Ana Ledo, referentes à Unidade Curricular "Estágio", apresentados à Faculdade de Farmácia da Universidade de Coimbra, para apreciação na prestação de provas públicas de Mestrado Integrado em Ciências Farmacêuticas.

Setembro de 2023

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Setembro de 2023

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Coimbra, 28 de agosto de 2023.

Luana Souto Rocha

(Luana Souto Rocha)

Agradecimentos

Ao concluir esta etapa, fica um sentimento nostálgico de momentos por que passei, e é com uma profunda gratidão e carinho que endereço os meus agradecimentos a todos aqueles que me acompanharam ao longo deste percurso de cinco anos.

À minha família, em particular aos meus pais, por todo o amor inabalável, apoio incondicional e motivação constante… Sem vocês não teria sido possível concretizar o meu sonho.

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Um obrigada nunca será suficiente para

exprimir a minha gratidão.

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Parte I

Relatório de Estágio – Bluepharma

Lista de Siglas e Acrónimos

DAG – Desenvolvimento Analítico e Galénico

EC – Estágio Curricular

EPI´S – Equipamentos de Proteção Individual

FFUC – Faculdade de Farmácia da Universidade de Coimbra

HPLC-GC – do inglês *High Performance Liquid Chromatography-Gas Chromatography*

IF – Indústria Farmacêutica

MICF – Mestrado Integrado em Ciências Farmacêuticas

RH – Recursos Humanos

SOP'S – *Standard Operating Procedures*

SWOT – do inglês *Strengths, Weaknesses, Opportunities and Threats*

1. Introdução

A Indústria Farmacêutica (IF) é uma das saídas profissionais que o Mestrado Integrado em Ciências Farmacêuticas (MICF) oferece e, tendo sido a que mais curiosidade me suscitou, candidatei-me ao estágio curricular (EC) na Bluepharma.

A Bluepharma foi a minha escolha por diversos motivos, o primeiro por ser uma empresa familiar, pela proximidade que sempre teve com a Faculdade de Farmácia da Universidade de Coimbra (FFUC). O segundo, porque sendo uma IF totalmente portuguesa, possui todos os departamentos intervenientes no ciclo do medicamento, desde a sua conceção até à sua produção e libertação para o mercado. O terceiro e último motivo, reside no fator curiosidade e interesse pela IF em si. Apesar de na FFUC sermos bem preparados para desempenhar funções numa IF, sinto que faltava o "ver como se faz" na realidade e como seriam os meus dias se trabalhasse nesta área.

Assim, candidatei-me, fui alvo de uma entrevista de estágio e fui selecionada para o departamento de Desenvolvimento Analítico e Galénico (DAG), no qual acompanhei um projeto de degradação forçada. A minha experiência foi desafiante e muito enriquecedora, uma vez que tive diversas formações, tive a oportunidade de desenvolver as minhas aptidões laboratoriais e contactar com o método *High Performance Liquid Chromatography-Gas Chromatography* (HPLC-GC), muito utilizado no DAG.

2. Contextualização

A Bluepharma é uma reconhecida IF sediada em São Martinho do Bispo, Coimbra. Nasceu em 2001 e, desde então, tem-se focado em fazer chegar o medicamento mais barato à população. Para isso, dedica-se diariamente à investigação, desenvolvimento e produção de medicamentos, particularmente de genéricos e biosimilares.[1]

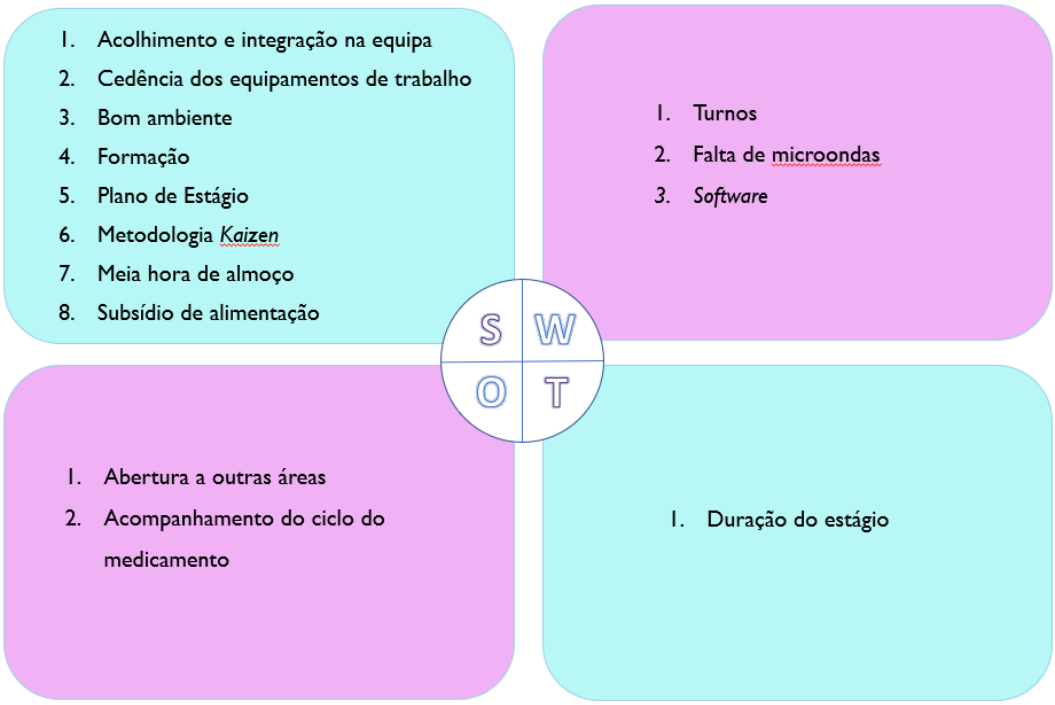
Além de Portugal, a Bluepharma está presente em Angola, Espanha, Estados Unidos da América e Moçambique, e exporta os seus produtos para o mercado internacional, tendo sido alvo de reconhecimento e distinções pelo seu trabalho de excelência e inovação. A empresa possui, ainda, parcerias de colaboração com instituições universitárias e centros de investigação no sentido de trazer terapias inovadoras para o mercado.[1]

2.1 Departamento de Desenvolvimento Analítico e Galénico

O DAG é composto pelo desenvolvimento analítico, desenvolvimento galénico e estabilidades. Foca-se essencialmente no desenvolvimento e validação de métodos analíticos que assegurem a qualidade, pureza, potência e a estabilidade dos fármacos dentro dos intervalos de referência.

3. Análise SWOT

Nesta secção segue-se uma análise relativa ao meu EC na Bluepharma, na qual abordo os pontos fortes, fracos, as oportunidades e ameaças que senti enquanto estagiária. Essa informação encontra-se resumida no Esquema 1 e, em mais detalhe, nos pontos seguintes.



Esquema 1: Análise SWOT do EC na Indústria Farmacêutica Bluepharma.

3.1 *Strengths* (Forças)

3.1.1 Acolhimento e integração na equipa

No primeiro dia, os estagiários da FFUC participaram numa sessão levada a cabo pelos Recursos Humanos (RH), na qual nos introduziram à Bluepharma, à sua organização e ao modo como nós, enquanto estagiários, deveríamos proceder desde o momento em que entramos na empresa até quando saímos.

Mais tarde, tivemos uma sessão de acolhimento ministrada pelo Presidente Paulo Barradas Rebelo, na qual nos contextualizou acerca da história da Bluepharma, de como esta

empresa nasceu, se desenvolveu e como se encontra em constante expansão em prol de fazer chegar o medicamento ao preço mais barato à população.

Ao nível do DAG, senti-me muito bem acolhida e integrada desde o primeiro até ao último dia. Houve um constante esforço e dedicação por parte dos meus colegas para que eu aprendesse e pudesse tirar o máximo de proveito do trabalho desenvolvido.

3.1.2 Cedência dos equipamentos de trabalho

O facto de a Bluepharma facultar os equipamentos de trabalho, como o computador portátil, rato, mochila e equipamentos de proteção individual (EPI´s), demonstra que há preocupação em que tenhamos todas as condições para que possamos trabalhar. O computador portátil foi particularmente importante para que me pudesse adaptar mais facilmente às dinâmicas adotadas pela empresa, nomeadamente ao nível da leitura de documentação necessária e da utilização da conta de *e-mail* profissional.

3.1.3 Bom Ambiente

O bom ambiente que se vive no DAG é indiscutível, pude presenciá-lo na primeira pessoa e, confesso, que é um fator determinante para que possamos desempenhar o trabalho com prazer.

Quando surgem obstáculos ao nível das tarefas desenvolvidas, os colaboradores ajudam-se mutuamente na tentativa de perceber qual o fator que pode estar a desencadear o problema e sentem-se genuinamente felizes quando tudo corre bem.

3.1.4 Formação

As formações facultadas pela Bluepharma, quer presenciais, quer *online*, demonstraram-se muito enriquecedoras e esclarecedoras. Deste modo, pude relembrar conceitos e matérias lecionadas na FFUC e aprender mais sobre a organização da empresa.

No final de cada formação, era submetida a um exame de conhecimentos com pontuação atribuível, no qual teria de ter uma nota mínima exigida (80% em 100%).

Para além das formações, foi-me exigida a leitura de *Standard Operating Procedures* (SOP´s) nas quais constam os procedimentos das diversas operações realizadas diariamente na empresa, mais concretamente e no meu caso, ao nível do DAG.

3.1.5 Plano de Estágio

Aquando da minha integração no estágio, foi-me facultada uma tutora que estaria responsável por mim – a Doutora Alexandra Gonçalves – que me explicou como seria o meu EC e me incumbiu certas tarefas, entre as quais o projeto no qual iria participar – as degradações forçadas.

Numa fase inicial, enquanto o projeto em causa não começava, fui acompanhando os meus colegas, nomeadamente o Joel Esteves, que me ensinou muito acerca do trabalho que desenvolvia no DAG. Desta forma, não só fui relembrando conceitos como fui absorvendo conhecimentos e pondo-os em prática a nível laboratorial.

Entretanto, tive a oportunidade de elaborar o protocolo laboratorial das degradações forçadas, tendo-se dado, posteriormente, início a esta atividade. De uma forma breve, as degradações forçadas consistem em sujeitar a formulação e a forma farmacêutica final a condições de degradação, tais como a hidrólise ácida, básica, humidade, calor, oxidação, entre outras, num determinado período de tempo, de forma a ocorrer uma degradação controlada dentro de intervalos previamente especificados e poder passar, assim, à etapa seguinte.

Desta forma, acompanhei a Maria Neves, a analista responsável por este projeto, que me transmitiu diversos conhecimentos relacionados com as degradações forçadas e com o desenvolvimento analítico em geral. A Maria Neves deu-me a oportunidade de desenvolver as minhas aptidões laboratoriais, capacidade de organização e de execução de tarefas em tempo limite.

3.1.6 Metodologia *Kaizen*

Diariamente, organizam-se reuniões *Kaizen* cujo intuito é o de informar a equipa e a coordenação do ponto de situação do trabalho desenvolvido por cada colaborador, sendo que no caso de não se conseguirem obter os resultados pretendidos ou de haver alguma discordância, se gera uma discussão construtiva de modo a poder solucionar determinados problemas/obstáculos.

Esta metodologia demonstrou ser muito enriquecedora para mim, enquanto estagiária, visto que para além de ter participado ativamente na mesma, pude trabalhar a minha capacidade de organização e comunicação.

3.1.7 Meia hora de almoço

Meia hora de almoço demonstrou ser suficiente para poder almoçar/jantar na cantina, socializar com os colegas e regressar às tarefas em execução e, assim, poder sair meia hora mais cedo. Tanto no turno diurno, como no noturno, essa meia hora surtiu efeito positivo.

3.1.8 Subsídio de alimentação

A Bluepharma faculta um cartão onde é depositado mensalmente o subsídio de alimentação, à semelhança do que acontece com os restantes colaboradores da empresa. Desta forma, é possível almoçar ou jantar na cantina com parte desse valor, sendo que o restante fica disponível para ser gasto em estabelecimentos aderentes, como os supermercados.

Sinto que este foi um fator importante para o meu EC, pela ajuda financeira inerente que constituiu.

3.2 *Weaknesses* (Fraquezas)

3.2.1 Turnos

Nos departamentos ligados ao laboratório, nomeadamente ao nível do DAG, os colaboradores executam turnos rotativos semanalmente. O turno da manhã tem início às 7h30 e fim às 16h, sendo que o turno da noite começa às 14h30, terminando às 23h.

Devido ao facto de os turnos serem rotativos, senti que me impactou negativamente, visto que as minhas rotinas de sono variavam semana após semana, além de que me sentia mais produtiva no horário da manhã do que no da noite.

Assim, considero que a existência de turnos com horários díspares foi uma fraqueza do meu EC.

3.2.2 Falta de microondas

A Bluepharma está equipada com uma cantina que serve almoços e jantares, com várias opções de ementa. Os colaboradores podem utilizar parte do subsídio de alimentação que recebem a cada mês para fazerem as suas refeições na cantina. Contudo, uma vez que nem todos os colaboradores vão à cantina, por diversos motivos, dos quais saliento as suas particularidades alimentares, vêem-se obrigados a comer a frio o que levam de casa.

Desta forma, considero que a existência de um microondas não iria prejudicar o funcionamento da cantina e da Bluepharma, sendo, por outro lado, uma grande ajuda para muitos colaboradores.

3.2.3 *Software*

Apesar de ter tido a oportunidade de observar o trabalho no *software* utilizado no DAG para análise de resultados e validações, sinto que o facto de não explorar por mim própria essa ferramenta, bastante complexa na minha opinião, constituiu um ponto fraco do meu EC.

3.3 *Opportunities* (Oportunidades)

3.3.1 Abertura a outras áreas

Ter feito EC no DAG ao longo de 3 meses foi uma excelente oportunidade para adquirir conhecimentos e me desenvolver. Além disso, foi fundamental para perceber se era um departamento com o qual me identificava, pelo que concluí com toda a certeza que sim. Contudo, fica-me a restar a curiosidade de saber como me sentiria nos restantes departamentos desta IF. Assim, considero pertinente a abertura a outras áreas de modo a que os estagiários possam experienciar de alguma forma como é trabalhar nos restantes departamentos.

Apesar do tempo de estágio ser relativamente curto, penso que uma semana dedicada a esta atividade não seria uma perda de tempo, mas sim, uma mais-valia.

3.3.2 Acompanhamento do ciclo do medicamento

No DAG, são desenvolvidos métodos analíticos que são fulcrais para o desenvolvimento do medicamento. Depois de finda esta etapa e de cumpridos os requisitos necessários, o produto passa à fase seguinte, o controlo de qualidade, e daí por diante no designado "ciclo do medicamento". A dada altura, o analista que desenvolveu aquele método e participou ativamente na conceção daquele produto, deixa de ter acesso à sua informação.

Desta forma, considero pertinente a desconstrução desta barreira, que poderá ser feita, por exemplo, através da inclusão do analista em reuniões ligadas ao medicamento em questão. A meu ver, este aspeto seria uma oportunidade na medida em que o analista não só se sentiria recompensado por ver o seguimento do seu trabalho, como também poderia ter voz ativa no solucionamento de problemas que possam surgir ao longo do ciclo do medicamento e que, muitas vezes, exigem o alinhamento do método previamente desenvolvido.

Enquanto estagiária, seria também uma oportunidade, visto que seria interessante presenciar o seguimento de um projeto.

3.4 *Threats* (Ameaças)

3.4.1 Duração do Estágio

Durante o meu EC na Bluepharma, adquiri muitos conhecimentos e senti uma grande progressão naquele que foi um curto intervalo de tempo - três meses. Contudo, não descartando a minha aprendizagem, considero que se tivesse mais tempo, poderia ter alargado o meu leque de conhecimentos, como por exemplo, aprender a trabalhar com o *software* computacional utilizado no DAG ou acompanhar outro tipo de projetos.

4. Considerações Finais

O EC na Bluepharma foi muito gratificante e não podia estar mais orgulhosa desta experiência. Graças a este EC, pude concluir que a IF, nomeadamente o DAG é, de facto, algo em que me revejo a trabalhar.

Para além de ter tido a oportunidade adquirir conhecimentos que me enriqueceram e que serão, certamente, úteis para o meu futuro enquanto profissional, pude fazê-lo num ambiente profissional, acolhedor e de espírito de entreajuda.

Assim, quero deixar o meu agradecimento especial a todos os que me acompanharam e contribuíram para a concretização deste EC com sucesso. A todos os colegas do DAG, nomeadamente à Maria e ao Joel, o meu muito obrigada por toda a formação, respeito e carinho. Sem dúvida, que sem a equipa maravilhosa que me acompanhou, o meu EC não teria sido a mesma coisa.

5. Referências Bibliográficas

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Parte II

Relatório de Estágio – Farmácia Gastromil

Lista de Siglas e Acrónimos

DT – Direção Técnica

EC – Estágio Curricular

FC – Farmácia Comunitária

LVMNSRM – Locais de Venda de Medicamentos Não Sujeitos a Receita Médica

MICF – Mestrado Integrado em Ciências Farmacêuticas

MICF – Mestrado Integrado em Ciências Farmacêuticas

MNSRM – Medicamentos Não Sujeitos a Receita Médica

PIM – Preparação Individualizada da Medicação

PVP – Preço de Venda ao Público

SWOT – do inglês *Strengths, Weaknesses, Opportunities and Threats*

VALORMED – Sociedade Gestora de Resíduos de Embalagens e Medicamentos

1. Introdução

O Mestrado Integrado em Ciências Farmacêuticas (MICF) dá-nos a possibilidade de trabalhar em Farmácia Comunitária (FC), sendo esta a saída profissional que emprega mais farmacêuticos em Portugal. Os farmacêuticos comunitários são os profissionais de saúde mais acessíveis do público. O aconselhamento, a dispensa de medicamentos, a revisão da terapêutica, o supervisionamento e a coordenação do trabalho dos restantes profissionais e colegas são algumas das inúmeras tarefas que um farmacêutico desempenha numa FC.

Ao longo da minha vida, sempre considerei o trabalho em farmácia comunitária interessante e algo que me imaginaria a exercer no futuro. Assim, durante os cinco meses de estágio em farmácia, incluindo o de verão e o curricular, permitiu-me experienciar este setor e adquirir diversas competências.

A Farmácia Gastromil foi a minha escolha não só por me permitir regressar a casa, ao fim de quase cinco anos em Coimbra, mas também por ser uma farmácia com muito boa reputação e acarinhada pelo público.

2. Contextualização da Farmácia Gastromil

A Farmácia Gastromil localiza-se no coração da cidade de Viseu, junto à zona Histórica. Conta com 84 anos de atividade, tendo utentes fidelizados desde o princípio do seu funcionamento.

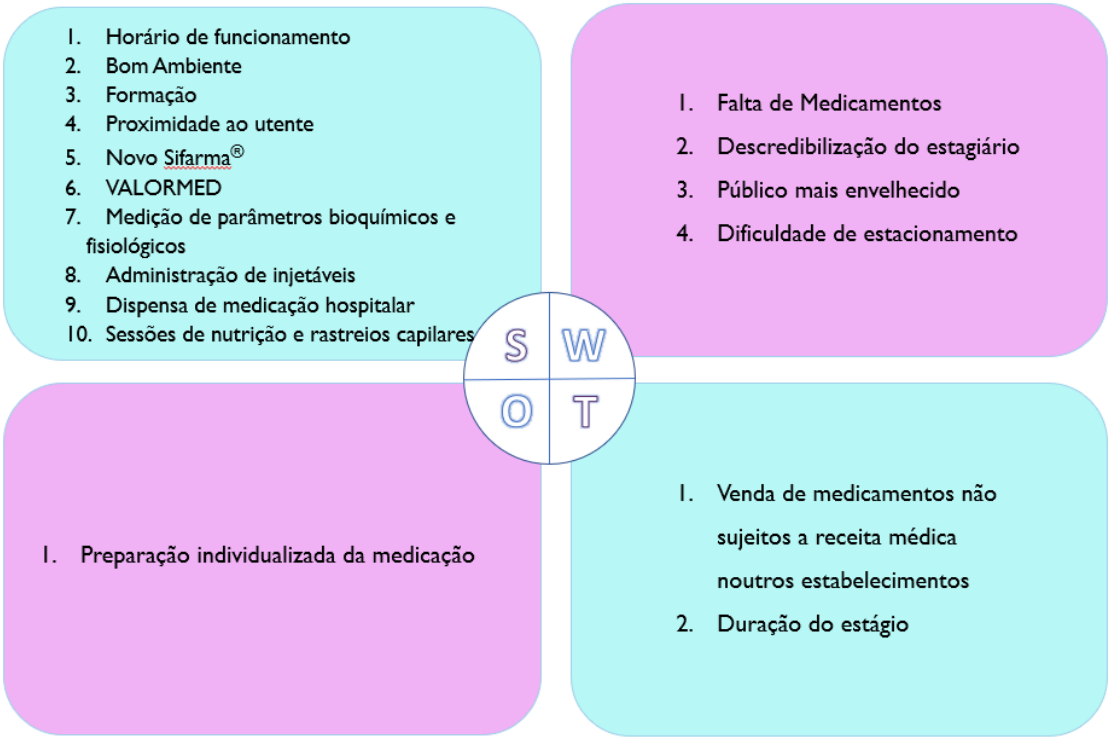
A Farmácia Gastromil é muito movimentada, tendo uma maior afluência de turistas que visitam a cidade, e de idosos, não só pelo facto de ser uma farmácia familiar à qual já estão ligados há vários anos, como também por este estabelecimento se encontrar junto a uma zona de comércio tradicional.

O horário de funcionamento é de segunda a sexta-feira, das 8h às 20h, e ao sábado das 8h às 19h.

A sua equipa é constituída por duas Farmacêuticas, a Dra. Alice Oliva e a Dra. Amália Alves, por uma Técnica Licenciada, a Margarida Santos, e dois Técnicos de Farmácia, o Francisco Silva e a Eduarda Simões. A Direção Técnica (DT) é ocupada pela Dra. Alice Oliva, também proprietária deste estabelecimento.

3. Análise SWOT

Nesta secção segue-se uma análise SWOT relativa ao meu EC na Farmácia Gastromil, na qual abordo os pontos fortes, fracos, as oportunidades e ameaças que senti enquanto estagiária. Essa informação encontra-se resumida no Esquema 2 e, em mais detalhe, nos pontos seguintes.



Esquema 2: Análise SWOT do EC na Farmácia Gastromil.

3.1 *Streangths* (Forças)

3.1.1 Horário de funcionamento

A realidade de muitas farmácias é a de funcionarem diariamente, inclusivamente aos domingos e em horários noturnos.

O horário de funcionamento da Farmácia Gastromil é de segunda a sexta-feira das 8h às 20h e aos sábados das 8h às 19h. Como tal, este horário revela-se muito positivo para os funcionários na medida em que não se trabalham noites, nem domingos.

3.1.2 Bom Ambiente

A Farmácia Gastromil destaca-se pela sua equipa dinâmica, bem-disposta e próxima ao utente. Enquanto estagiária, pude integrar-me neste bom ambiente, presenciar o profissionalismo de cada funcionário e aprender com todos.

3.1.3 Formação

Ao longo do EC, fui sendo alvo de formação pela minha orientadora de estágio, a Dra. Amália, que me transmitiu os conhecimentos necessários ao trabalho em farmácia comunitária. Desta forma, adquiri ferramentas ao nível do aconselhamento terapêutico e da gestão da farmácia.

Os restantes colegas foram imprescindíveis na medida em que sempre se prontificaram a esclarecer qualquer dúvida que eu tivesse.

Tive, também, a oportunidade de assistir a inúmeras formações online e presenciais no âmbito do aconselhamento terapêutico em FC e apresentação de novos produtos por parte de alguns laboratórios.

Toda a formação que tive foi útil para adquirir ferramentas essenciais ao desempenho de tarefas ligadas à gestão e ao aconselhamento em FC.

3.1.4 Proximidade ao utente

Os utentes que entram na Farmácia Gastromil são carinhosamente atendidos, sendo a maioria idosos fidelizados há muitos anos. Desta forma, desenvolve-se um clima de familiaridade com estas pessoas, o que faz com que se sintam em casa e queiram voltar onde são bem recebidos.

Este contacto revelou-se muito positivo para mim, pois permitiu desenvolver a minha capacidade de comunicação e de relação com o próximo.

3.1.5 Novo Sifarma®

O Sifarma® é um dos diversos *softwares* utilizados pelas farmácias a nível nacional, sendo este o mais usado. O Sifarma 2000® é o mais antigo e, gradualmente, a Glint tem feito a transição para o novo modelo – o Novo Sifarma®. Contudo, como pude constatar numa formação presencial da Glint sobre como trabalhar no Novo Sifarma®, não tem sido fácil para as farmácias se adaptarem a este novo *software*.

Na Farmácia Gastromil, já se utiliza praticamente na totalidade o novo modelo, o que constituiu uma mais-valia para mim. Para além disso, também fui ensinada a trabalhar com o Sifarma 2000®, visto que ainda é utilizado, nomeadamente no desempenho de determinadas tarefas em que o novo modelo ainda está a ser alvo de atualizações.

O facto de saber executar tarefas em ambos os sistemas, constitui um ponto forte do meu EC.

3.1.6 VALORMED

A Farmácia Gastromil está equipada com um contentor VALORMED que tem como finalidade a gestão de resíduos de medicamentos e embalagens fora de validade e/ou de uso.[1]

Enquanto estagiária, pude sensibilizar as pessoas para os benefícios desta prática, tendo verificado uma alta adesão, o que demonstra a consciencialização das pessoas para esta temática.

3.1.7 Medição de parâmetros bioquímicos e fisiológicos

A medição da pressão arterial, glicémia, colesterol e triglicéridos são serviços que a farmácia dispõe. Uma vez que muitos utentes os solicitam para controlar a sua saúde, pude acostumar-me a estas práticas, executando-as regularmente. Além destes serviços, os utentes tinham, ainda, a possibilidade de controlarem o seu peso e altura através da balança e medidor de altura automáticos.

3.1.8 Administração de injetáveis

Tive a oportunidade de experienciar a administração de injetáveis na farmácia e apesar de ser necessário um curso para o efeito, o facto de visualizar e de me explicarem como se faz, permitiu-me estar mais familiarizada com esta prática.

3.1.9 Dispensa de medicação hospitalar

A dispensa de medicação hospitalar em contexto de FC começou a ser uma realidade, motivada pela situação pandémica recentemente experienciada. Assim, facilitou-se a vida dos utentes na medida em que se evitaram deslocações ao hospital para levantar a sua medicação.

O registo e a dispensa destes medicamentos são efetuados ao nível do Sifarma®, sendo que o facto de ter presenciado esta funcionalidade, permitiu-me alargar o meu leque de conhecimentos relacionados com o programa e com a medicação hospitalar. [2]

3.1.10 Sessões de nutrição e Rastreios capilares

Adicionalmente, a farmácia possui sessões de nutrição associadas à dieta EasySlim® e promove rastreios capilares a par com a Advancis Capilar®. Deste modo, pude ser parte ativa na promoção destas atividades e percecionar como serviços não farmacêuticos podem complementar e enriquecer as farmácias em Portugal, tendo como ponto fulcral a saúde dos utentes.

3.2 *Weaknesses* (Fraquezas)

3.2.1 Falta de medicamentos

A falta de medicamentos nas farmácias é uma realidade que se deve múltiplos fatores, entre os quais o aumento da inflação, a maior dependência, ao nível da produção, de países como a China e a Índia, e a exportação para o estrangeiro.

Alguns medicamentos deixaram de ser comercializados, enquanto outros tinham fases em que esgotavam e outros que chegavam à farmácia em quantidades residuais, dificultando o acesso ao medicamento.

O facto de ter vivenciado o desespero dos utentes, sentir impotência de os conseguir ajudar e lidar com alguma incompreensão, foi algo negativo para mim.

3.2.2 Descredibilização do Estagiário

Durante o meu EC, apesar de na grande maioria das vezes os utentes confiarem nos meus conhecimentos ao nível do aconselhamento e dispensa de medicação, houve situações em que tal não aconteceu. Assim, apesar de estar sob supervisão da minha orientadora de estágio, senti um certo estigma por ser estagiária e alguma insegurança por parte das pessoas quando preferiam ser atendidos por outro elemento da equipa.

3.2.3 Público mais envelhecido

Como referi anteriormente, grande parte do público da Farmácia Gastromil é envelhecido, o que impactou positiva e negativamente o meu estágio. Por um lado, fez com que desenvolvesse competências para lidar com esta faixa etária e estivesse mais ocorrente das patologias que a assolam, bem como da medicação que tomam. Por outro lado, sinto que o facto de o público jovem ser mais escasso, nomeadamente crianças e bebés, constituiu um ponto fraco do meu EC, visto não ter experienciado tanto esta realidade.

3.2.4 Dificuldade de estacionamento

O estacionamento próximo da Farmácia Gastromil é escasso e praticamente inexistente, o que leva a que os funcionários e os utentes tenham dificuldades e se vejam obrigados a deixar o carro longe do estabelecimento. Eu própria passava por isso diariamente e, portanto, para além de ser um ponto negativo para a farmácia/utentes, foi também para mim.

3.3 *Opportunities* (Oportunidades)

3.3.1 Preparação individualizada da medicação

Na minha opinião, seria pertinente a Preparação Individualizada de Medicação (PIM) no sentido de facilitar a vida aos idosos e evitar erros nas tomas, que acontecem por múltiplos fatores, muitas vezes associados à confusão mental e ao analfabetismo. Contudo, esta é uma questão transcendente à farmácia visto que se trata de um serviço caro que a maioria dos utentes não está disposto a pagar e, portanto, deveria ser alvo de apoio pelo Estado.

3.4 *Threats* (Ameaças)

3.4.1 Venda de Medicamentos Não Sujeitos a Receita Médica noutros estabelecimentos

A venda de Medicamentos Não Sujeitos a Receita Médica (MNSRM) em Locais de Venda de Medicamentos Não Sujeitos a Receita Médica (LVMNSRM), como as parafarmácias e os hipermercados, constitui uma ameaça. Atualmente, as pessoas estão mais informadas devido ao que vêem na *Internet* e, por isso, tendem a ser mais independentes no ato da compra. Contudo, nem sempre estão bem informadas, e é aí que as farmácias se distinguem com o aconselhamento farmacêutico.

Além deste fator, há um outro relacionado com o Preço de Venda ao Público (PVP). Como os hipermercados e certas parafarmácias compram em grandes quantidades, conseguem obter descontos maiores que aqueles que as farmácias conseguiriam, visto que não adquirem nessas quantidades, tornando-se difícil competir com os preços praticados nestes estabelecimentos.

O cartão das farmácias portuguesas surgiu para contrariar esta situação, incentivando as pessoas a deslocarem-se às farmácias e fidelizá-las. Contudo, apesar destes esforços, é certo que as farmácias perderam muito com a perda de exclusividade de venda destes produtos.[3]

O facto de as pessoas preferirem comprar MNSRM em LVMNSRM e de descartarem o aconselhamento farmacêutico constituiu uma ameaça do meu EC.

3.4.2 Duração do estágio

O EC na Farmácia Gastromil permitiu-me adquirir ferramentas, complementá-las às que apreendi ao longo do curso e colocá-las em prática. Contudo, atendendo à sua curta duração, considero que ficaram situações por experienciar e conhecimentos por cimentar.

4. Casos Práticos

O EC na Farmácia Gastromil representa uma etapa crucial no meu percurso académico e na minha transição para o mundo farmacêutico. Assim, tive a oportunidade de ampliar os meus conhecimentos e adquirir diversas competências que serão, certamente, necessárias para a minha futura carreira enquanto farmacêutica.

Os casos práticos que se apresentam são um exemplo de situações reais que experienciei em contexto de FC e que me permitiram aplicar conceitos teóricos que apreendi ao longo dos cinco anos do MICF.

Caso Prático 1:

Um utente de 65 anos, visivelmente abatido, entra na farmácia, procurando tratamento para as dores que sente nos joelhos e que pioram ao fim do dia. Aconselhei a toma do suplemento alimentar Artrozen®, um a dois comprimidos ao dia, que com a sua fórmula concentrada em glucosamina, condroitina, colagénio, ácido hialurónico, quercetina e silício ajudam no alívio da dor e inflamação articular. Recomendei, ainda, o gel Fisiocrem® à base de substâncias naturais, como a arnica, hipericão, calêndula e menta que ajudam no alívio imediato da dor. Por último, aconselhei a toma do Nurofen Musc®, que contém 400 mg ibuprofeno para reduzir a dor e a inflamação, com posologia de um comprimido a cada 8 horas, em caso de necessidade.[4,5,6]

Caso Prático 2:

Uma utente de 31 anos chega à farmácia e pede antibiótico para tratar uma infeção urinária que suspeita ter. Referi que não podia dispensar antibiótico sem receita médica e questionei qual a sua sintomatologia e a duração, pelo que me respondeu que urinava frequentemente, sentia ardor ao urinar, que a sua urina apresentava um odor fétido e que era o segundo dia em que se senti assim. Desta forma, concluí que era muito provável tratar-se de uma infeção urinária.

Aconselhei, portanto, Advancis Urivial SOS® em ampolas, com uma formulação concentrada de plantas tradicionais, tais como uva-ursina, urze, bétula, urtiga-maior e cavalinha, além da vitamina B6, que reforça o sistema imunitário no combate à infeção. Recomendei duas tomas ao dia, ao fim do pequeno-almoço e do jantar, diluindo a ampola num copo de água.

Aconselhei, também, a toma da Infusão Farline® trato urinário, com cavalinha e herniária, referindo que podia utilizar até duas saquetas por dia.

Para além do aconselhamento farmacológico, referi uma série de medidas não farmacológicas no sentido de resolver e/ou evitar infeções urinárias futuras, tais como: beber cerca de 1,5L de água por dia, evitar o uso de pensos higiénicos diários, não passar muito tempo sem ir à casa de banho, optar por roupa de algodão e mais larga e limpar da vagina para o ânus de modo a evitar o transporte de microorganismos do trato gastrointestinal para a uretra.

Ressalvei, ainda, a importância de que na persistência destes sintomas deveria consultar o médico.[7,8]

Caso Prático 3:

Um senhor de 40 anos vai à farmácia Gastromil na tentativa de procurar resolver o seu problema. O senhor tinha uma ferida no dedo médio que resultou de um pequeno acidente enquanto realizava trabalhos manuais no dia anterior. A ferida tinha um aspeto infecionado pelo que aconselhei o uso do spray Diaseptyl Ducray® que com o seu elevado poder bactericida e antissético, permite limpar e desinfetar a lesão sem irritar e o uso da pomada Polisulfadê® que apresenta antibiótico – bacitracina – e cicatrizante na sua composição, 2 vezes ao dia. Para além disso, recomendei material de penso ao utente para proteger a ferida de agentes ambientais. [9,10]

Caso Prático 4:

Uma utente de 20 anos dirigiu-se à farmácia com queixas de secura e prurido vaginal, procurando algo que acalmasse e resolvesse o problema. Referiu ter esta sintomatologia há uma semana, sendo que já era recorrente sentir-se assim. Questionei se costumava usar gel lavante adequado e hidratante íntimo, ao qual me respondeu que apenas recorria à hidratação vulvar e que se sentia melhor quando a usava.

Neste sentido, aconselhei um gel lavante íntimo delicado Lactacyd®, com uma fórmula adequada para uma mucosa sensível e facilmente irritável, e um creme hidratante íntimo vulvar da ISDIN®, que ajuda a aliviar a irritação, a secura e o prurido. Para além destes, aconselhei, ainda, o suplemento alimentar Floradela®, que contém *Lactobacilus reuteri* e *Lactobacilus rhamnosus*, cujo objetivo é o de restabelecer a microbiota vaginal.

No ato da dispensa, expliquei que a posologia recomendada era de duas aplicações do creme na região vulvar, duas vezes por dia; duas cápsulas do suplemento por dia e que a lavagem com o gel seria diária.[12,13]

Duas semanas mais tarde, a utente voltou à farmácia, afirmando sentir-se muito melhor e agradeceu toda a atenção e o aconselhamento dispensados.

Caso Prático 5:

Um utente de 25 anos chega à farmácia e diz estar a ter episódios de diarreia. Questionei há quanto tempo se sentia assim e se tinha vómitos e/ou febre, ao que ele me respondeu 2 dias e apenas diarreia intensa, respetivamente. Desta forma, aconselhei a toma de um probiótico, o Prolif®, contendo *Saccharomyces boulardii*, e de um antidiarreico, o Lenodiar®, constituído à base de Actitan-F e de substâncias naturais como flavonóides e taninos. Recomendei, ainda, a toma de Dyoralite®, que contém glicose, cloreto de sódio, cloreto de potássio e citrato dissódico, no sentido de corrigir as alterações hidroeletrolíticas decorrentes deste tipo de cenários.

Relativamente à posologia, recomendei a toma de uma cápsula de prolif três vezes ao dia, de duas cápsulas de Lenodiar®, duas a três vezes ao dia, conforme a gravidade do distúrbio, e uma saqueta de Dyoralite® após dejeção diarreica. Ressalvei, também, a importância de ingestão de água para não ficar desidratado.[14,15,16]

Duas semanas depois, o utente vem à farmácia e revela que a medicação que dispensei surtiu efeito positivo, tendo resolvido o problema.

5. Considerações Finais

O EC na Farmácia Gastromil foi muito gratificante em todos os aspetos, dos quais saliento o ambiente, a formação e o profissionalismo. Foi-me dada a possibilidade de crescer enquanto ser humano e futura farmacêutica e não posso estar mais feliz depois da conclusão deste EC.

Um agradecimento do fundo do coração aos meus colegas sempre bem-dispostos… À Dra. Amália, pelos conhecimentos que me transmitiu e por toda a preparação para estar apta a "desempenhar funções em qualquer farmácia", foi incansável… Ao Francisco, à Margarida, à Eduarda e à Dra. Alice, por toda a ajuda e carinho que tiveram comigo desde o primeiro dia.

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Parte III

Monografia

"The relationship between Cholesterol and Alzheimer´s disease"

Abstract

This dissertation addresses the complex relationship between Alzheimer's disease and cholesterol. This purpose is accomplished by examining the Alzheimer's disease pathophysiology, exploring cholesterol involvement through the three distinct pathways of cholesterol formation (endogenous, exogenous, and neurological), elucidating the role of the Apolipoprotein E4 allele, as the main cholesterol-related genetic risk factor on late-onset Alzheimer's disease, and evaluating the influence of statins, as cholesterol-lowering agents, in this disease.

The Apolipoprotein E4 allele constitutes a significant focal point of this review in a way that details the diverse molecular processes that underlie the impact of this allele on cholesterol metabolism and, consequently, its effect on Alzheimer's disease susceptibility. Another prominent aspect of this dissertation is the role of statins on Alzheimer's disease and its multifactorial effects. While some evidence suggests potential benefits of this cholesterol-lowering medications in reducing Alzheimer's disease risk, others do not find positive effects, and yet another perspective highlights the negative consequences of excessively lowering cholesterol levels.

Thus, this thesis synthesizes current research findings providing a nuanced perspective on the intricate relationships linking Alzheimer's disease, cholesterol, and statins, and addressing areas where knowledge is lacking.

Keywords: Alzheimer´s disease; Beta-Amyloid; Cholesterol; APOE4 allele; Statins.

Resumo

Esta dissertação aborda a complexa relação existente entre a doença de Alzheimer e o colesterol. O propósito deste trabalho é cumprido, na medida em que se analisa a fisiopatologia da doença de Alzheimer, se explora o envolvimento do colesterol através das três vias distintas de formação do colesterol (síntese endógena, exógena e neurológica), se elucida o papel do alelo E4 da Apolipoproteína E, como sendo o principal fator de risco genético, ligado ao colesterol, sobre a doença de Alzheimer de início tardio, e se avalia a influência das estatinas nesta doença, como agentes redutores de colesterol.

O alelo E4 da Apolipoproteína E constitui um ponto significativo desta revisão, uma vez que são detalhados os diversos processos moleculares subjacentes ao impacto do mesmo no metabolismo do colesterol e, consequentemente, o seu efeito sobre a doença de Alzheimer. Outro aspeto proeminente desta dissertação é o papel relevante das estatinas sobre a doença de Alzheimer e os respetivos efeitos multifatoriais. Enquanto algumas evidências sugerem potenciais benefícios destes medicamentos na redução do risco de doença de Alzheimer, outras não encontram efeitos positivos, sendo que numa outra perspetiva, se destacam, ainda, as consequências negativas resultantes da redução excessiva dos níveis de colesterol.

Assim, esta monografia sintetiza os resultados de investigações atuais, proporcionando uma perspetiva elaborada sobre as relações complexas existentes entre a doença de Alzheimer, o colesterol e as estatinas, abordando campos onde o conhecimento ainda é escasso.

Palavras-chave: Doença de Alzheimer; Amilóide-Beta; Colesterol; Alelo APOE4; Estatinas.

Lista de Siglas e Acrónimos

24-OHC – 24-Hydroxycholesterol

25-OHC – 25-Hydroxycholesterol

27-OHC – 27-Hydroxycholesterol

7-KC – 7-Ketocholesterol

7α-OHC – 7α-Hydroxycholesterol

7β-OHC – 7β-Hydroxycholesterol

AA – Arachidonic Acid

ABCA1 – ATP-Binding Cassette Transporter A1

ABCA7 – ATP-Binding Cassette Transporter A7

ABCG1 – ATP-Binding Cassette Transporter G1

ACAT – Acyl-CoA:Cholesterol Acyltransferase

Acetyl-CoA – Acetyl Coenzyme A

AD – Alzheimer's Disease

AP-1 – Activator Protein-1

APOA-I – Apolipoprotein A-I

APOB – Apolipoprotein B

APOB-100 – Apolipoprotein B-100

APOB-48 – Apolipoprotein B-48

APOC – Apolipoprotein C

APOC-I – Apolipoprotein C-I

APOC-II – Apolipoprotein C-II

APOC-III – Apolipoprotein C-III

APOE – Apolipoprotein E

APOE2 – Apolipoprotein E2 Allele

APOE3 – Apolipoprotein E3 Allele

APOE4 – Apolipoprotein E4 Allele

APOJ – Apolipoprotein J

APP – Amyloid Precursor Protein

Aβ – Beta-Amyloid

Aβ42-GFP – Amyloid Beta 42-Green Fluorescent Protein

BACE – β-Secretase

BACE1 – β-Secretase 1

BBB – Blood-Brain Barrier

BDNF – Brain-Derived Neurotrophic Factor

cFos – FBJ Osteosarcoma

CH25H – Cholesterol 25-Hydroxylase

CNS – Central Nervous System

cPLA2 – Calcium-Dependent Cytosolic Phospholipase A2

CSF – Cerebrospinal Fluid

CYP – Cytochrome P450

CYP27A1 – Cholesterol 27-Hydroxylase

CYP46A1 – Cholesterol 24-Hydroxylase

CYP7A1 – Cholesterol 7a-Hydroxylase;

DHCR24 – 24-Dehydrocholesterol Reductase

DLK – Dual Leucine-Zipper Kinase

DMAPP – Dimethylallyl Diphosphate

FAD – Familial Alzheimer's Disease

FFAs – Free Fatty Acids

flAPP – Full-length APP

HDL – High-Density Lipoprotein

HIF-1α – Hypoxia Inducible Factor 1α

HMG-CoA – 3-Hydroxy-3-Methylglutaryl-Coenzyme A

HMGCR – 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase

IDL – Intermediate Density Lipoproteins

IL-1β – Interleukin-1β

IL-6 – Interleukin-6

iNOS – Inducible Nitric Oxide Synthase

IPP – Isopentenyl Pyrophosphate

LCAT – Lecithin-Cholesterol-Acyltransferase

LDL – Low-Density Lipoproteins

LDL-C – Low-Density Lipoprotein Cholesterol

LDLR – Low-Density Lipoprotein Receptor

LNC – Lipid-Core Nanocapsules

LPL – Lipoprotein Lipase

LRP1 – Low-Density Lipoprotein Receptor-Related Protein 1

LRP1 – Low-Density Lipoprotein Receptor-Related Protein 1

LTB4 – Leukotriene B4

LV-LNC – Lovastatin-Lipid Core Nanocapsules

MVA – Mevalonate

MCI – Mild Cognitive Impairment

NCs – Polymer Nanocapsules

NFT – Neurofibrillary Tangles

NPC1L1 – Niemann-Pick C1-Like 1

PGE2 – Prostaglandin E2

P-gp – P-glycoprotein

PK – Pyruvate Kinase

pro-BDNF – Precursor of Brain-Derived Neurotrophic Factor

PSEN1 – Presenilin-1

PSEN2 – Presenilin-2

pTau – Hyperphosphorylated Tau

RCTs – Large Randomized Controlled Trials

ROS – Reactive Oxygen Species

SAD – Sporadic Alzheimer's Disease

sAPPα – Soluble Amyloid Precursor Protein α

sAPPβ – Soluble Amyloid Precursor Protein β

SORL1 – Sortilin-Related Receptor

SR-BI – Scavenger Receptor Class B Type I receptor

SV-LNC – Simvastatin- Lipid Core Nanocapsules

TAG – Triacylglycerols

TCA – Tricarboxylic Acid Cycle

TNFα – Tumor Necrosis Factor-α

TREM-2 – Triggering Receptor Expressed on Myeloid Cells 2

VLDL – Very Low-Density Lipoproteins

αCTF – α C-Terminal Fragment

β -CTF – β C-terminal Fragment

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder that affects millions of individuals across the globe, resulting in memory loss, cognitive decline, and eventually severe disability and mortality. Although the precise causes of Alzheimer's disease are not completely understood, research suggests there may be a link between cholesterol metabolism and the risk of developing the disease.

Cholesterol is a type of lipid that is necessary for numerous physiological processes, including the formation of cell membranes and the production of hormones. However, excessive cholesterol levels in the blood can increase the risk of heart disease, stroke, and other health issues. Recent research indicates that elevated cholesterol levels may also be associated with an increased risk of AD through the contribution of underlying processes that lead to Beta-Amyloid (Aβ) plaque formation in the brain, which are characteristic of the disease.

Statins are a class of medications extensively used to reduce the level of cholesterol in the blood and thus the risk of cardiovascular disease. Statins function by inhibiting the liver enzyme 3-Hydroxy-3-Methyl-Glutaryl-CoA Reductase (HMGCR), which is responsible for cholesterol production. In addition to their cholesterol-lowering effects, some studies suggest that statins may also have neuroprotective properties and could potentially reduce the risk of AD. Nonetheless, the evidence for the efficacy of statins in preventing or treating AD remains limited and controversial, with some studies indicating a protective effect and others finding no benefits.

This dissertation aims to investigate the relationship between cholesterol, statins, and AD, with a particular focus on the main risk factor for sporadic AD – Apolipoprotein E4 allele (APOE4) – and the potential function of statins in reducing AD risk. We will review the current literature on the epidemiology, pathophysiology, and risk factors of AD and investigate the mechanisms by which cholesterol and statins may affect the pathogenesis of AD. In addition, we will investigate the evidence for the efficacy and safety of statin therapy in the prevention and treatment of AD, as well as the potential hazards and benefits of statins.

Thus, this thesis attempts to improve understanding of the relationship that exists between AD, cholesterol, and statins by combining current research and addressing knowledge gaps.

2. Neurological diseases

2.1 Physiopathology of Alzheimer's disease

Alzheimer's disease (AD) is the most common type of dementia.[1] It is a multifactorial, chronic, age-dependent neurodegenerative disorder [2] that is characterized by the pathological accumulation of Hyperphosphorylated Tau (pTau), which causes intracellular Neurofibrillary Tangles (NFT), and Aβ, which causes extracellular Senile Plaques (SNP).[13]

Alzheimer's disease (AD) has clearly been linked to an increase in the amount Aβ in the brain. Healthy individuals produce Aβ through β- and γ-secretase- mediated cleavage of the amyloid precursor protein (APP); however, under certain conditions, the molecule accumulates to above optimal concentrations, resulting in self-aggregation of monomeric peptides into neurotoxic assemblies. Aβ peptides accumulate extracellularly in result of the aggregation and fibrillation of Aβ peptides that have been released from neurons. Tau pathology, on the other hand, is the result of intracellular fibrillation of pTau into NFT.[4] While Aβ generation has been extensively studied, the mechanisms underlying pTau accumulation in AD remain inadequately understood.[5]

In addition to these two defining histopathological hallmarks, numerous studies suggest that lipid metabolism plays a role in the development and progression of the disease. The presence of aberrant lipid granules in neuroglia has been noted since the initial observations reported by Alois Alzheimer himself.[6]

Genetically, AD can be subdivided into two subgroups: Familial Alzheimer's disease (FAD) with early onset and Sporadic Alzheimer's disease (SAD) with late onset[7] — the most common. All familial AD mutations occur in genes that code for Aβ-generating proteins, such as APP, Presenilin-1 (PSEN1) and Presenilin-2 (PSEN2), catalytic subunits of the secretase complex, resulting in enhanced amyloidogenic processing. In contrast, the quantification of the rates of Aβ synthesis and clearance within the Cerebrospinal Fluid (CSF) indicates that Aβ accumulation in sporadic Alzheimer's disease (AD) patients is primarily due to significant defects in the peptide's clearance.[7] Sporadic AD is the most prevalent form of AD (>95%) and typically manifests after age 65 – indeed, age is the most relevant risk factor for SAD.[8]

There are currently no effective treatments that can reliably reduce the onset of AD or alleviate, delay, or halt the disease's clinical progression. At present, the primary prevention of AD focuses on the modulation of cardiovascular risk factors, such as dyslipidaemia, blood pressure, glucose at fasting, or weight.[9]

3. Lipid metabolism

3.1 Cholesterol synthesis

Cholesterol is an essential component of all cell membranes, and it is found mostly in animals. It is a steroid alcohol composed of 27 carbon atoms arrayed in a tetracyclic sterane ring with a C-H side chain.[10]

Although cholesterol can be consumed through animal products present in the diet, most of the cholesterol is synthesized endogenously, with nearly 90% of its synthesis taking place in the liver and intestine. The majority of peripheral cells rely on the exogenous delivery of cholesterol by lipoproteins.[10]

Cholesterol is produced by the liver through a series of enzymatic reactions (Figure 1). The transformation of Acetyl Coenzyme A (acetyl-CoA) into 3-Hydroxy-3-Methylglutaryl-Coenzyme A (HMG-CoA) is the initial phase. In the second phase, HMG-CoA is converted into Mevalonate (MVA), which is then converted into isoprene units - isopentenyl diphosphate (IPP) and Dimethylallyl Diphosphate (DMAPP) - resulting in the formation of squalene, the precursor to cholesterol. In the third and final step, which takes place in the endoplasmic reticulum, squalene is converted to lanosterol, which then endures further modifications to form cholesterol.[10]

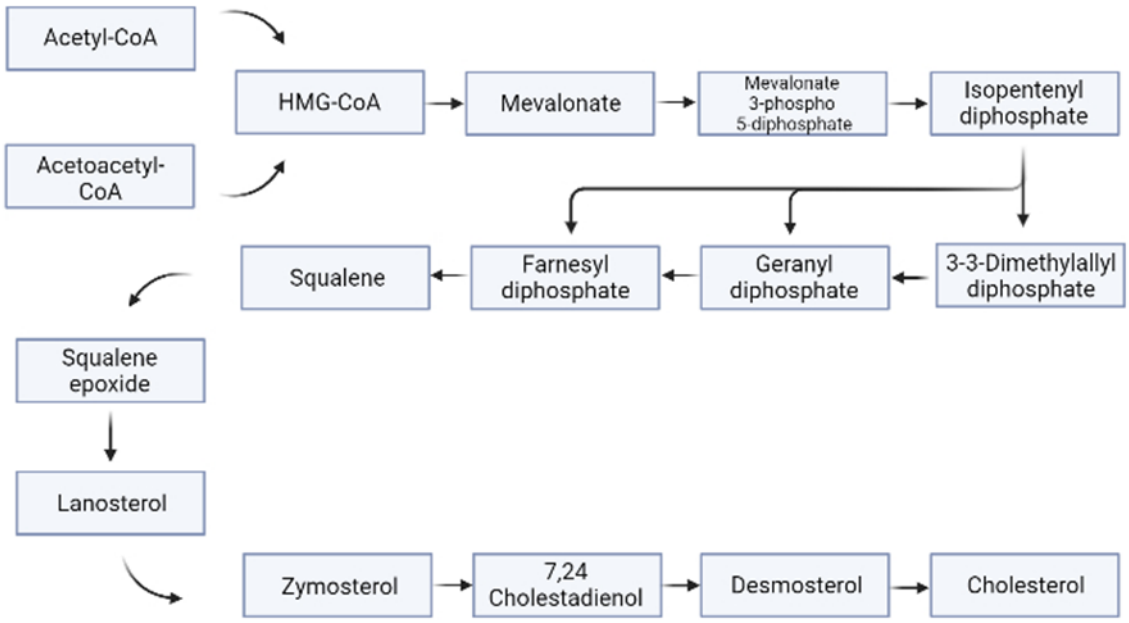

Figure 1: Cholesterol byosinthesis. (Adapted from [10]).

3.1.1 Exogeneous pathway

The exogenous pathway is responsible for transporting dietary lipids from the intestine to the liver and peripheral cells.[10]

As shown in Figure 2, it begins in enterocytes' endoplasmic reticulum, where chylomicrons are synthesized from dietary fats. After passing through the lymphatic system, the chylomicrons enter the bloodstream. ***(1)*** The chylomicron contains the apolipoproteins B-48 (APOB-48), E (APOE), and C-II (APOC-II) (Table 1). ApoC-II activates lipoprotein lipase (LPL) in the capillaries of adipose tissue, the heart, skeletal muscle and the breast gland during lactation, allowing the release of Free Fatty Acids (FFAs) to these tissues. Therefore, chylomicrons transport dietary fatty acids to the tissues, enabling the transport of dietary fatty acids for utilization or storage ***(2)***.[11]

Chylomicron remnants, containing cholesterol, APOE, and APOB-48, continue through the bloodstream to the liver. Receptors in the liver bind APOE to chylomicron remnants and regulate their absorption via endocytosis. ***(3)*** These remnants release their cholesterol and are degraded into lysosomes in the liver. ***(4)*** This route is referred to as the exogenous pathway (red arrows in Figure 2).[11]

When the diet contains more fatty acids and cholesterol than is required for immediate use as fuel or as precursors for other molecules, the liver converts them into Triacylglycerols (TAG) or cholesterol esters and packages them, forming Very Low-Density Lipoproteins (VLDL) with specific apolipoproteins. ***(5)*** Additionally, excess carbohydrates in the diet can become VLDL which contain, along with TAG and cholesterol esters, APOB-100, APOC-I, APOC-II, APOC-III and APOE (Table 1). VLDL travels through the bloodstream to muscle and adipose tissue.[11]

3.1.2 Endogeneous pathway

The endogenous cholesterol pathway involves the synthesis, transport and use of cholesterol in the body, with the liver playing a pivotal role in regulating cholesterol levels and preserving its homeostasis.[10]

ApoC-II activates LPL in muscle and adipose tissue capillaries, catalysing the release of fatty acids from VLDL triacylglycerols. Adipocytes store these fatty acids as TAG in lipid vesicles, whereas myocytes primarily oxidises these fatty acids for energy. When insulin levels are elevated (post-meal), VLDL transport dietary lipids to fat tissue for storage. During fasting or between meals, the fatty acids used to produce VLDL in the liver are primarily derived from adipose tissue, targeting myocytes and skeletal muscle. A portion of VLDL turns into

intermediate Density lipoproteins (IDL), from which TAG is removed to generate Low-Density Lipoproteins (LDL). ***(6)***[11]

Low-Density lipoproteins, rich in cholesterol/cholesterol esters, contains APOB-100 as its primary apolipoprotein, and distributes cholesterol to extra-hepatic tissues. The plasma membrane of these tissues contains receptors that recognize APOB-100, which facilitate the uptake of cholesterol and cholesterol esters. ***(7)*** Low-Density lipoproteins also transport cholesterol to macrophages, occasionally transforming them into foam cells. **(*8*)** LDL receptors of hepatocytes capture peripheral tissue-unabsorbed LDL, enabling cholesterol to be added to membranes, changed into bile acids, or storage as lipid droplets in the cytosol via Acyl-CoA:cholesterol Acyltransferase (ACAT). ***(9)***[11]

This constitutes the endogenous pathway of cholesterol metabolism and transport. (blue arrows in Figure 2).

3.1.3 Reverse Transport of Cholesterol

Reverse cholesterol transport involves High-Density Lipoprotein (HDL) removing excess cholesterol from peripheral tissues, such as the arterial wall, and transporting it to the liver for excretion.[12]

The HDL particles originate in the liver and small intestine as small protein-rich particles with minimal cholesterol and no cholesterol esters. **(*10*)** HDL contains APOA-I and other apolipoproteins, alongside lecithin-cholesterol-acyltransferase (LCAT), an enzyme that catalyses the formation of cholesterol esters from lecithin or phosphatidylcholine (PC) and cholesterol. The LCAT on the surface of emerging HDL particles starts cholesterol ester formation, transforming the disc-shaped emerging HDL into a mature spherical HDL molecules. **(*11*)** Emerging HDL can also acquire cholesterol from cholesterol-rich cells (including macrophages and foam cells). **(*12*)** Mature HDL is then returned to the liver, expelling cholesterol via the Scavenger Receptor Class B type I Receptor (SR-BI). ***(13)*** The cholesterol ester carrier protein transfers some HDL cholesterol esters to LDL, and HDL transports cholesterol in the opposite direction. (yellow arrows in Figures 2).[11]

The liver converts the majority of this cholesterol into bile salts, which are then deposited in the gallbladder. During meals, bile salts are secreted into the intestine, where they disperse larger fat fragments into micelles that can be digested by lipases. Bile salts are reabsorbed by the liver ***(14)*** and recirculated via the bile vein in entero-hepatic circulation (green arrows in Figure 2).[11]

The release of sterol into the liver and other tissues via the SR-BI does not involve endocytosis, the mechanism used for LDL capture. In contrast, when HDL binds to SR-BI receptors on hepatocytes or steroid-like tissues, such as the adrenal gland, these receptors selectively transfer cholesterol and other lipids from HDL to cells. The released HDL recirculates into the bloodstream to extract additional lipids from the remaining chylomicron, VLDL, and cholesterol-overloaded cells.[11]

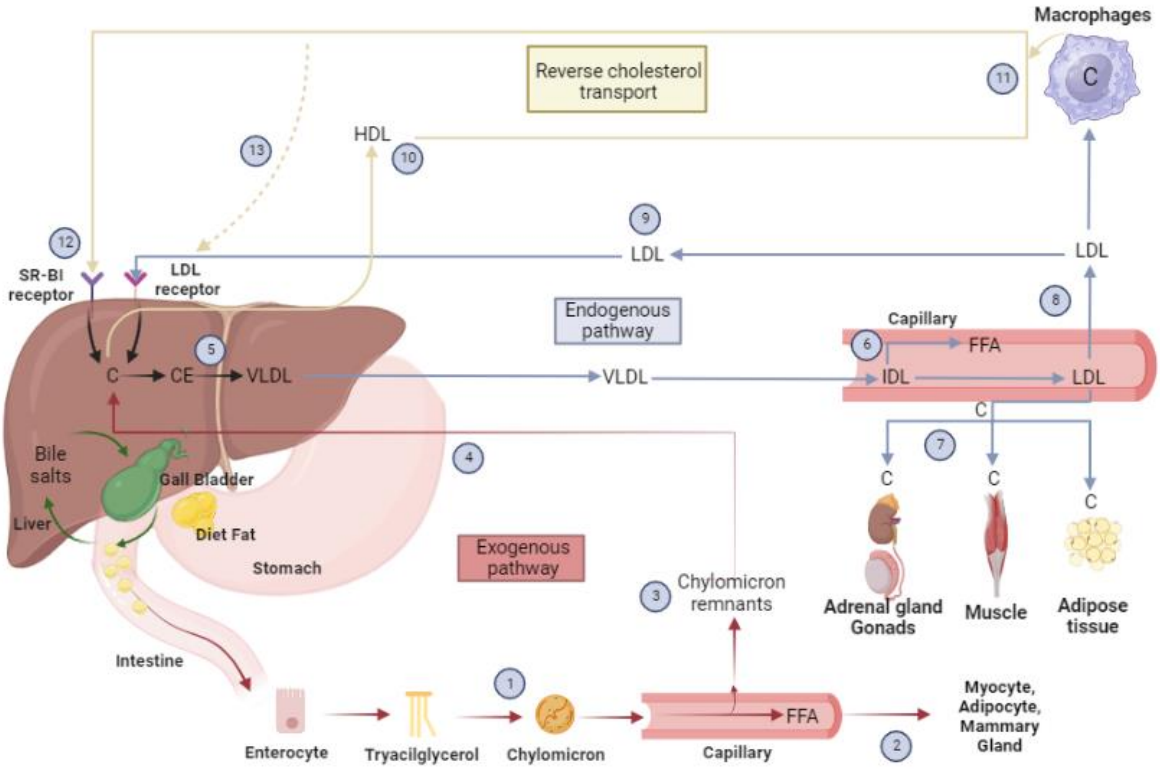

Figure 1: Exogenous, Endogenous and Reverse transport of cholesterol. In the exogenous pathway (red arrows), dietary lipids are packed into chylomicrons. Crossing capillaries, LPL liberates most TAG in fat and muscle tissues. The Liver capture the remaining chylomicrons, while liver-produced bile salts, disperse dietary fats, reabsorbed in the entero-hepatic pathway (green arrows). In the endogenous pathway (gray arrows), liver-synthesized/packaged lipids migrate to tissues by VLDL. Extracting lipids from VLDL converts part of the VLDL into LDL, transporting cholesterol to tissues/liver. Liver captures LDL, VLDL remnants (IDLs) and chylomicron remains. Excess cholesterol in tissues returns to the liver via HDL in the reverse cholesterol transport. (yellow arrows). C: cholesterol; CE: esters of cholesterol; SR-BI: Scavenger Receptor Class B Type I receptor; LDL: Low density lipoprotein; VLDL: Very-low density lipoprotein; IDL: Intermediate-density lipoprotein; HDL: High-density lipoprotein; FFA: Free fatty acids. (Adapted from [11]).

3.1.4 The Neurological pathway

Cholesterol is needed for the development and maintenance of neuronal physiology, and 25% of the body's cholesterol is found in brain tissue.[13] Cholesterol metabolism is a complex and tightly controlled process that includes biosynthesis, transport, esterification, and efflux.[14] Brain cholesterol homeostasis is highly regulated to maintain steady levels of the sterol, and it

is almost entirely independent from that of the periphery due to the blood–brain barrier (BBB).[3]

Both astrocytes and neurons are capable of synthesizing cholesterol from acetyl-CoA via HMGCR. However, as the neurons mature, astrocytes and glial cells provide the necessary cholesterol to neurons in the form of lipid-rich APOE particles.[13] Figure 3 highlights that early in the biosynthesis process of cholesterol, acetyl-CoA is converted into lanosterol, the first sterol. In this stage, HMGCR is the first rate-limiting enzyme that helps turn HMG-CoA into MVA, which starts a chain of reactions that leads to the formation of lanosterol. Then, in the post-lanosterol stage, sterol intermediates follow either the "Bloch" or "Kandutsch–Russell" pathway, both of which lead to cholesterol synthesis. The enzyme 24-Dehydrocholesterol Reductase (DHCR24), that is expressed in various tissues, including the brain, can act on any "Bloch pathway" intermediate and redirect it to the "Kandutsch–Russell pathway".[3]

For brain cholesterol homeostasis, excess cholesterol is esterified, stored, or oxidized into oxysterols. The primary oxysterol involved in this process is 24-Hydroxycholesterol (24-OHC), also known as cerebrosterol, which is derived from the oxidation of cholesterol by cholesterol 24-Hydroxylase (CYP46A1), a cytochrome P-450 enzyme expressed primarily by neurons but also by astrocytes. The 24-OHC crosses the BBB into the systemic circulation (99%) and is subsequently metabolized in the liver; less than 1% of the 24-OHC enters the CSF. Other oxysterols, such as 27-Hydroxycholesterol (27-OHC), 25-Hydroxycholesterol (25-OHC) and 7α-Hydroxycholesterol (7α-OHC), can be generated enzymatically or through cholesterol auto-oxidation.[3]

Mature neurons in the adult brain depend mostly on astrocytic supply of cholesterol. Once cholesterol is synthesized by astrocytes, it is loaded into lipoproteins similar to HDL that contain APOE and then secreted into the extracellular space by ATP-Binding Cassette Transporter A1 (ABCA1) and ATP-Binding Cassette Transporter G1 (ABCG1) (Table 2). Lipoproteins are then transported to neurons, where they are internalized by the Low-Density Lipoprotein Receptor (LDLR) and Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1) receptors located on the membrane of nerve cells. APOE is recycled after endocytosis, and cholesterol is used for cell membrane turnover and repair, dendritic growth, myelin formation, synaptogenesis, and neurotransmitter release.[3]

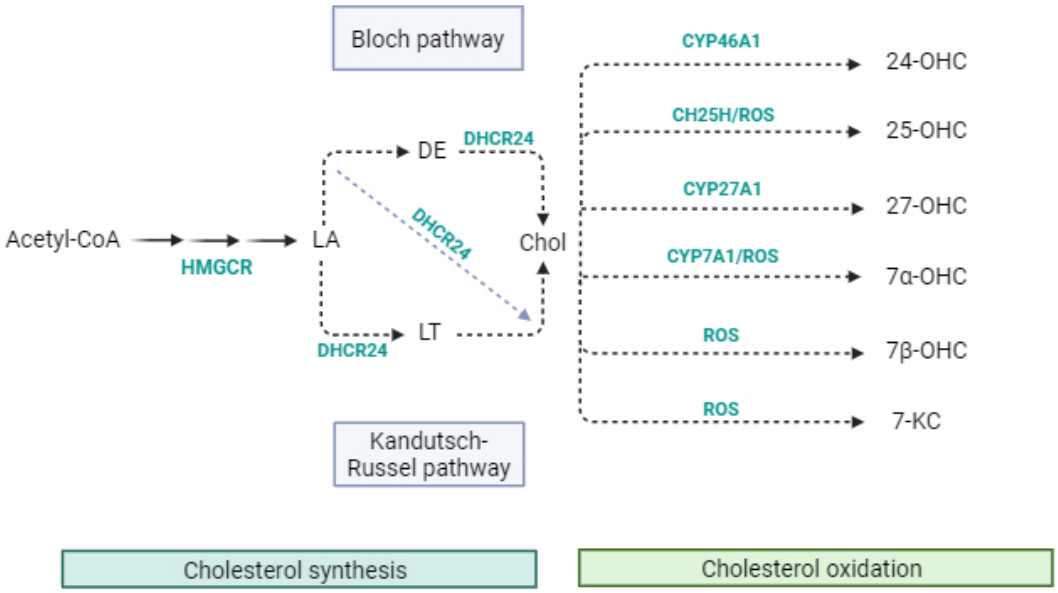

Figure 3. Neurological pathway - The HMGCR enzyme converts acetyl-CoA to lanosterol during early cholesterol biosynthesis. Cholesterol is produced from lanosterol via the "Bloch" or "Kandutsch–Russell" pathways. In the brain, the DHCR24 enzyme can convert "Bloch" to "Kandutsch–Russell" intermediates. The CYP46A1 enzyme stores or oxidizes excess brain cholesterol to oxysterols, predominantly 24-OHC. The majority of 24-OHC exits the brain for the liver, with some entering the CSF. Other oxysterols, such as 27-OHC, 25-OHC, and 7α-OHC, are produced enzymatically or via cholesterol oxidation. Acetyl-CoA: Acetyl coenzyme A; Chol: Cholesterol; CH25H: Cholesterol 25-hydroxylase; CYP46A1: Cholesterol 24-hydroxylase; CYP27A1: Cholesterol 27-hydroxylase; CYP7A1: Cholesterol 7a-hydroxylase; DE: Desmosterol; DHCR24: 24-dehydrocholesterol reductase; HMGCR: 3-hydroxy-3-methylglutaryl-CoA reductase; LA: Lanosterol; LT: Lathosterol; ROS: Reactive oxygen species; 7-KC: 7-ketocholesterol; 7α-OHC: 7α- hydroxycholesterol; 7β-OHC: 7β-hydroxycholesterol; 24-OHC: 24-hydroxycholesterol; 25-OHC: 25- hydroxycholesterol; 27-OHC: 27-hydroxycholesterol. (Adapted from [3]).

Tables I and II summarize the tissue/cellular localization and function of apolipoproteins and transporters involved in lipid metabolism as mentioned above, as well as the risk factors each one represents.

Table I: Apolipoproteins involved in lipid metabolism. APOB-100: Apolipoprotein B-100; APOB-48: Apolipoprotein B-48; APOE: Apolipoprotein E; APOJ: Apolipoprotein J; APOC: Apolipoprotein C; APOC-I: Apolipoprotein C-I; APOC-II - Apolipoprotein C-II; APOC-III: Apolipoprotein C-III. [11,15, 12, 16, 17]

Apolipoprotein	Localization	Function	Risk Factor
APOB - **APOB-100** - **APOB-48**	- APOB-100 is the predominant protein of LDL. - ApoB-48 is the predominant protein of chylomicrons.	- It serves a crucial role in lipid metabolism by preserving the structural integrity of lipoproteins. - Ligand for the LDL receptor, facilitating the uptake of cholesterol-rich LDL particles by cells.	- Atherosclerosis (it involves initial fatty streak deposition, followed by progression to complex atherosclerotic lesions that are susceptible to plaque rupture or endothelial erosion).
APOA1	- Main component of HDL.	- Responsible for reverse cholesterol transport from tissues to the liver and cholesterol excretion by promoting efflux from other tissues. - Activates LCAT. - Interact with ABC transporters.	- Premature coronary artery disease, hepatosplenomegaly and recurrent peripheral neuropathy in HDL deficiency. - Mutations in the human APOA1 gene induce amyloidosis, an inherited generalized amyloidosis.
APOE	- At CNS, primarily produced by astrocytes and to a lesser extent by microglia and neurons. - Component of the major lipoproteins in the periphery, including chylomicrons, VLDLs and HDLs.	- It induces VLDL clearance and remains of chylomicrons. - Primary apolipoprotein responsible for the transport of cholesterol in the brain. - Crucial role in neuronal maintenance and repair (e.g., synaptic homeostasis and axonal regeneration).	- Cardiovascular diseases and AD.
APOJ	- One of the minor HDL apolipoprotein components; - Widely distributed throughout the body, including CNS.	- Involved in a variety of physiological functions, related to neuroprotection and neurodegeneration (e.g, regulation of lipid transport and the elimination of misfolded proteins).	- Cardiovascular diseases and neurodegenerative diseases, such as AD (not fully understood).
APOC - **APOC -I** - **APOC-II** - **APOC-III**	- APOCIII is primarily found in chylomicrons and VLDL and, to a lesser extent, in LDL and HDL particles. - APOCIII is produced predominantly in the liver, and is also synthesized in the brain.	- Regulation of lipid metabolism. - APOC-II activates LPL. - APOC-III inhibits LPL.	- Increased incidence of cardiovascular disease and type 2 diabetes. - Progression of cardiovascular disease by modulating triglyceride metabolism and exerting direct atherogenic effects. - Proinflammatory effects on endothelial cells and monocytes. - Associated with cognitive function, dementia, and AD.

Table II: Transporters involved in lipid metabolism. ABCA1: ATP-Binding Cassette Transporter A1; ABCG1: ATP-Binding Cassette Transporter G1; ABCA7: ATP-Binding Cassette Transporter A7; SR-BI: Scavenger Receptor Class B Type 1 receptor; NPC1L1: Niemann-Pick C1-Like 1.[11,3,18,6,19]

Transporters	Localization	Function	Risk Factor
ABCA1 **ABCG1**	- ABCA1 is expressed in diverse tissues, such as liver, intestine, macrophages, adrenal glands and peripheral tissues. - ABCG1 is expressed in macrophages, endothelial cells, adipocytes and hepatocytes.	- Participant in cholesterol efflux and lipoprotein metabolism. - Utilize ATP hydrolysis to transport a diverse array of molecules against their concentration gradients, thereby contributing to the maintenance of the brain's microenvironment. - Give the BBB a crucial role in regulating brain cholesterol levels. - ABCA1 mediates the secretion of nascent discoidal APOE-rich particles from astrocytes; these nascent lipoproteins are further enriched in cholesterol and phospholipids by the activity of ABCG1, expressed in both astrocytes and neurons. This lipid enrichment, along with the activity of remodeling enzymes such as LCAT, results in the maturation of nascent particles into mature particles that deliver cholesterol to neurons by	- Atherosclerosis and cardiovascular disease.

		interacting with particular APOE receptors, such as the LDL receptor and its family members.	
ABCA7	- Expressed in multiple tissues, including the brain.	- Involved in lipid metabolism and transport. Mediates the production of HDL-like particles that are smaller and contain less cholesterol. - Endogenous ABCA7 modulate the host defence system, including phagocytic cell function.	- Mutations have been associated with an increased risk of developing AD.
SR-BI	- Expressed in the liver, adrenal glands and steroidogenic tissues.	- Role in reverse cholesterol transportation. - Promotes the delivery of cholesterol to the liver by promoting the uptake of cholesterol esters from HDL particles.	- Cardiovascular diseases (not well understood).
NPC1L1	- Primarily expressed in the small intestine	- Facilitates cholesterol absorption into intestinal cells from the intestinal lumen.	- None. - (Ezetimibe is a npc1l1 inhibitor and it reduces the absorption of cholesterol from diet).

4. Cholesterol relevance at CNS

Lipid metabolism, which includes cholesterol, oxysterols, fatty acids, and phospholipids, is implicated in several neurodegenerative diseases, such as AD. Cholesterol is an essential component of plasma membrane bilayers, where it influences structural and physical functions that include fluidity, curvature, and rigidity.[3] It is crucial for plasma membrane regionalization, myelin sheath formation, signal transduction, and synaptic formation and maintenance.[9] It is also involved in transmembrane signalling, membrane trafficking, endocytosis, and ion transport and regulates the functions of membrane proteins. Cholesterol also plays a role in the biosynthesis of bile acids and steroid hormones in the plasma membrane, both of which play crucial functional and biological functions as signal transducers.[20]

Even though the CNS accounts for only 2.1% of body weight, it contains 23% of total body cholesterol. Synaptic transmission is controlled by cholesterol in neurons and astrocytes.[20] Thus, cholesterol homeostasis in the normal brain is rigorously regulated to maintain steady-state cholesterol levels in the brain, which are essential for neuronal function and brain development. The maintenance of brain cholesterol homeostasis is essential for neuronal function, and anomalous brain cholesterol metabolism is linked to neurodegenerative diseases such as AD.[3] Therefore, an extraordinarily low cholesterol level has the potential to impair neurocognitive function. The Framingham Heart Study revealed that a certain cholesterol level is necessary to maintain normal cognitive performance.[21] Therefore, it is probable that the reduction of Low-Density Lipoprotein Cholesterol (LDL-C) to varying levels is associated with either cognitive impairment or enhancement, and achieving a balance of LDL-C is crucial for preserving cognitive function.[9]

The Brain-Blood Barrier (BBB) separates nearly all of the cholesterol in the brain from the cholesterol in the periphery. It does not imply, however, that dietary cholesterol cannot affect cholesterol metabolism in the CNS. Several side-chain hydroxylated metabolites of cholesterol, such as 24-OHC and 27-OHC, can cross the BBB. These hydroxycholesterols are both involved in neuroinflammation.[22]

Chronic hypercholesterolemia is associated with a variety of diseases, such as atherosclerosis, coronary heart disease, diabetes, and stroke. In addition, hypercholesterolemia may lead to neuroinflammation, which increases the risk of age-related neurological disorders such as AD. A 30-year retrospective cohort study revealed that hypercholesterolemia in midlife is associated with a 27% increased risk of dementia in old age. However, due to age-related cholesterol loss, the aging brain has an increased demand for cholesterol. Consequently, a cholesterol-rich diet in the elderly may have a protective influence on brain function, which opposes its negative effect, particularly on neuroinflammation. Epidemiological evidence supports this hypothesis by showing that hypercholesterolemia has no effect on dementia and cognitive decline in the elderly and that a low serum cholesterol level in elderly populations increases the risk of AD.[22]

The evidence suggesting serum cholesterol levels are a risk factor for dementia and cognitive decline is inconclusive, in part because different classes of lipoprotein have diverse or even opposing regulatory roles in inflammation and oxidative stress in the peripheral and Central Nervous Systems (CNS). Cholesterol is, then, necessary for the maintenance of normal brain functions, including signal transduction, synaptic plasticity, and memory formation.[22]

5. The link between cholesterol and AD risk

5.1 The impact of APOE4 isoform

The APOE4, a protein involved in lipid metabolism and cholesterol homeostasis, is the most significant known genetic risk factor for SAD.[23] Apolipoprotein E is predominantly produced by astrocytes in the brain in normal physiological conditions, however, neurons also produce APOE under stress.[24] This apolipoprotein is predominantly expressed in the liver and midbrain. Apolipoprotein E mRNA is expressed and distributed differently on distinct types of hippocampal neuronal cells and this difference is age-dependent in mice.[25]

Apolipoprotein E4 secreted by neurons is more neurotoxic than APOE4 from astrocytes. Neuronal APOE4 causes the onset of AD through a variety of pathophysiological

mechanisms, such as: stimulating microglia to induce neuroinflammation; directly leading to neuronal degeneration; participating in additional signaling pathways; mediating the production and aggregation of Aβ and Tau; preventing the excretion of lipids and resulting in lipid accumulation within cells. It can also reduce the development of myelin sheaths by interfering with the transport of cholesterol in neurons, thus inducing AD pathology.[26,27,28]

In humans, there are three polymorphic alleles of the APOE gene: E2, E3, and E4. These alleles occur at varying frequencies, with E3 occurring 65–70% of the time, followed by E4 and E2. In contrast, the frequency of the E4 allele is approximately 40% higher in AD patients compared to healthy individuals. Population studies have shown that the E4 allele is associated with a substantially increased risk of AD, whereas the E2 variant appears to have protective effects relative to E3, and this results from differences in the amino acid sequences of the APOE protein (Figure 4).[23]

Even though the association between APOE alleles and AD has been well established, the mechanisms underlying these risk differences remain poorly understood. [23]

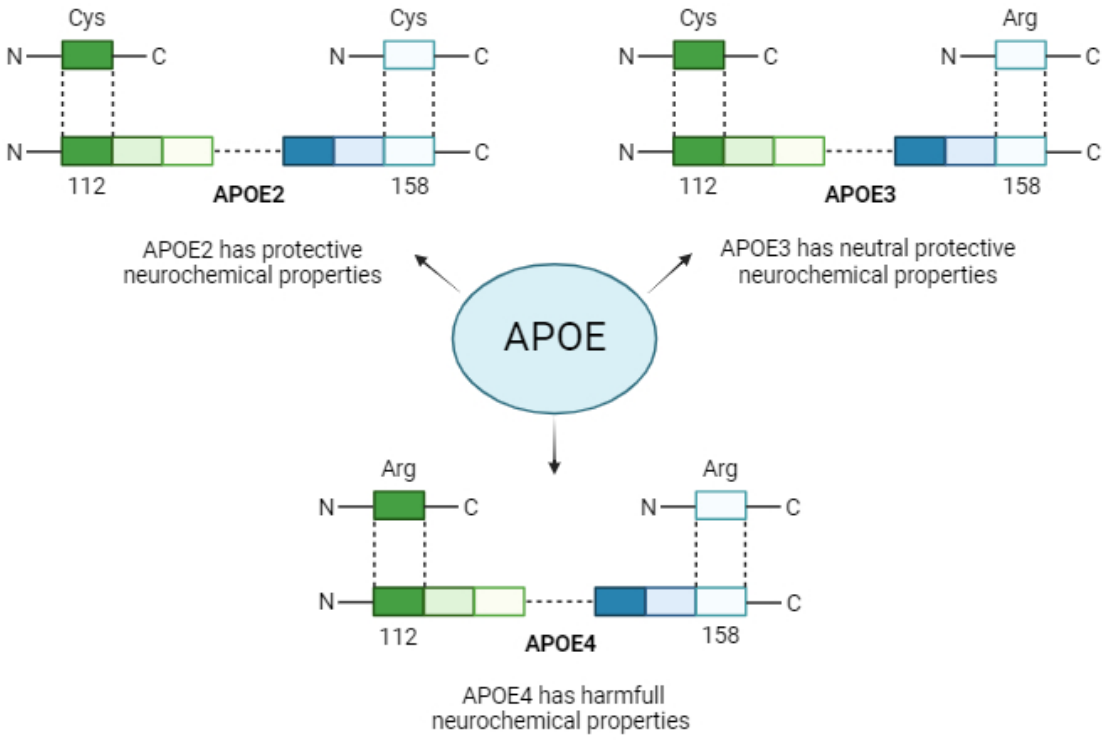

Figure 4. APOE isoforms and their neuronal functions. APOE contains three isoforms: E2, E3, and E4. The distinction between the three is the amino acid at positions 112 and 158. APOE2 contains at both locations and has a neuroprotective effect. APOE3 contains Cys at position 112 and Arg at position 158, which conserve neurons between E2 and E4. APOE4 is composed of arginine at both locations. Arg: Arginine; Cys: Cysteine; APOE: Apolipoprotein E; APOE2: Apolipoprotein E2; APOE3: Apolipoprotein E3; APOE4: Apolipoprotein E4. (Adapted from [23]).

5.1.1 The Link between APOE4 and neuroinflammation

The APOE protein has been identified to play a role in modulating the innate immune system and its isoform APOE4, has been associated with pro-inflammatory changes in both the central nervous system and periphery. Post-mortem brain samples expressing APOE4 show increased levels of inflammatory response proteins compared to APOE3 and APOE2-expressing tissues. Additionally, APOE isoforms control the survival or toxicity of neurons. Apolipoprotein E4 negatively impacts synaptogenesis, increases tau phosphorylation and neurotoxicity, and decreases astrocyte phagocytosis of synapses.[23]

To investigate the role of the APOE variants on neuroinflammation and neurotoxicity, researchers treated human microglia, astrocytes and neurons with APOE2/3/4 *in vitro* and evaluated the effects of these APOE isoforms on neuroinflammatory and neurotoxic markers. The findings demonstrated that APOE modulated microglial and astrocyte pro-inflammatory activity in an isoform-specific manner and influenced neuron survival through direct and indirect mechanisms. In addition, the study found that APOE4 increased the production of pro-inflammatory cytokines – such as Tumor Necrosis Factor-α (TNFα), Interleukin-1β (IL-1β) and Interleukin-6 (IL-6) - in both astrocytes and microglia, suggesting its potential role in affecting AD (Figure 5). The results also showed that APOE2 increased the secretion of mature brain-Derived Neurotrophic Factor (BDNF), which is protective against AD, whereas APOE3 and APOE4 increased the secretion of precursor of Brain-Derived Neurotrophic Factor (pro-BDNF), which is associated with cell mortality.[23]

One study demonstrated that APOE4 is associated with immunometabolic alterations in the brain, specifically in microglia. In their study using mice, the researchers investigated the role of APOE in relation to age, neuroinflammation, and AD pathology.[29] Microglia enriched with APOE4 exhibited distinct immunometabolic changes, including increased expression of Hypoxia Inducible Factor 1α (HIF-1α) and disruption of the Tricarboxylic Acid Cycle (TCA) (Figure 5). The protein HIF-1α is involved in the cellular response to low oxygen levels, and its increased expression in microglia enriched with APOE4 can suggest altered hypoxia responses. The TCA cycle is a metabolic system involved in energy biosynthesis and production, and its impairment in APOE4-expressing microglia suggests potential disruptions in cellular function and metabolism.[29]

In addition, ApoE4 may intensify microglial inflammatory response in the presence of amyloid plaques and impact the metabolism of lipids, which are essential for the integrity of cell membranes and the brain.[29]

WANG *et al.* reports that APOE4 genotype is also associated with increased activation of the Calcium-dependent Cytosolic Phospholipase A2 (cPLA2) signaling system. It increases inflammation and oxidative stress, through the release of Arachidonic Acid (AA), a precursor for the synthesis of various pro-inflammatory molecules, including Leukotriene B4 (LTB4), Reactive Oxygen Species (ROS) and increased expression of inducible nitric oxide synthase (iNOS) (Figure 5). These effects can be relevant in the onset and progression of SAD, in which inflammation and oxidative stress play a significant role. Increased activation of cPLA2 was observed in primary astrocytes, mice and human brain samples from APOE4 carriers.[30]

Triggering Receptor Expressed on Myeloid Cells 2 (TREM-2) is a microglia-expressed immune receptor that controls the expression of genes implicated in cholesterol transport and metabolism, including APOE.[31] To characterize the APOE-TREM2 interaction, ZHENHUA *et al.* demonstrated that the binding energy between APOE and TREM2 is isoform-dependent, with APOE4 being more potent than APOE3 and APOE2.[31] Emerging evidence identifies TREM2 as a APOE receptor, suggesting that interactions between APOE, specifically APOE4 and TREM2, might influence the development of AD (Figure 5). According to the study LI *et al.*, APOE4 causes neuroinflammation by either blocking the TREM2 pathway or activating the proinflammatory Prostaglandin E2 (PGE2) pathway (Figure 5). Nonetheless, this molecular interaction requires further study.[32]

Using *in vitro* AD mouse models, FITZ *et al.* observed that APOE4 is linked to a less effective microglial response to Aβ when compared to APOE3.[33] In contrast to APOE4, APOE3 lipoproteins displayed a more favourable response, characterized by rapid microglial migration towards Aβ, increased uptake of Aβ, and a reduction of Aβ-induced cognitive effects. These results show how important APOE isoforms are in controlling microglial behaviour and how they can interact with Aβ. The less efficient response of APOE4-enriched microglia may have implications for Aβ clearance and subsequent neuroinflammatory processes.[33]

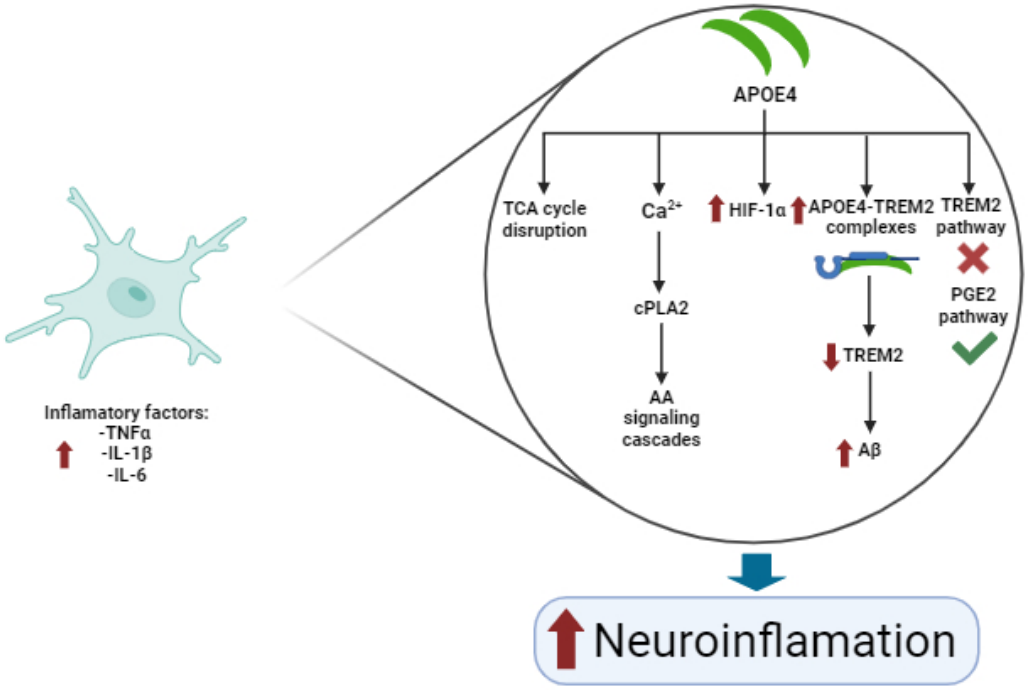

Figure 5. The APOE4 effects on neuroinflammation in AD. ApoE4 increases inflammatory factors, such as TNF-α, IL-1β and IL-6. ApoE4 can contribute to neuroinflammation through several mechanisms: disrupting the TCA cycle; activating cPLA2 and altering AA signaling pathways; increasing HIF-1α factor; influencing Aβ clearance through the interaction of ApoE4 and TREM2; activating the pro-inflammatory PGE2 pathway and inhibiting the anti-inflammatory TREM2 pathway. APOE4: Apolipoprotein E4; TNFα: Tumor Necrosis Factor-α; IL-1β: Interleukin-1β; IL-6: Interleukin-6; TCA: Tricarboxylic acid cycle; cPLA2: Calcium-dependent cytosolic phospholipase A2; AA: Arachidonic acid; HIF-1α: Hypoxia inducible factor 1α; TREM2: Tricarboxylic acid cycle Triggering Receptor Expressed on Myeloid Cells 2; Aβ: Beta-amyloid; PGE2: Prostaglandin E2.

5.1.2 The association between ApoE4 and Aβ aggregation

The metabolism and elimination of Aβ in the normal brain are mediated by APOE. APOE plays a crucial role in this process by directly interact with Aβ to form stable complexes that can be observed in senile plaques and NFTs, hallmark pathological features of AD.[34] The accumulation of Aβ, however, might be exacerbated by APOE4 because it can potentially interfere with normal clearance process, leading to increase Aβ aggregation and deposition, which contributes to the development and progression of AD.[34]

The observations made by LIN *et al.* reinforce the notion that APOE4 has a significant impact on astrocyte- and microglia-mediated Aβ clearance, leading to AD. It revealed several differences between APOE4 and APOE3 neurons. Neurons expressing APOE4 exhibited more synapses and secreted higher levels of Aβ42, a specific form of Aβ associated with AD, compared to neurons expressing APOE3. Besides, APOE4 neurons exhibited impaired Aβ uptake and increased cholesterol accumulation.[35]

Furthermore, this study also found that APOE4 microglia-like cells, which play a crucial role in clearing Aβ and maintaining brain health, had altered morphologies and a diminished capacity to phagocytose Aβ, which contributes to AD. However, these researchers also demonstrated that converting APOE4 to APOE3 in brain cells derived from SAD-induced Pluripotent Stem Cells (iPSCs) attenuated multiple AD-related processes.[35]

Using flow cytometry, KARA *et al.* also reported a significant interaction between APOE4 and Aβ in primary immortalized astrocytes when compared to APOE2 and APOE3, which increases the possibility of protein deposition complex formation.[36]

In the presence of Aβ, APOE, depending on its specific subtype, can act as a signaling molecule. APOE4 has a greater tendency to bind to APOE receptors than APOE2 and APOE3 (Figure 6). This enhanced binding results in the interaction of Dual Leucine-zipper Kinase (DLK) with APOE receptors. This interaction activates Extracellular Signal-Regulated Kinase 1 and 2 Mitogen-Activated Protein Kinase (ERK1/2 MAP kinase) and phosphorylates cellular FBJ Osteosarcoma (cFos), a protein involved in gene regulation, which stimulates the transcription factor Activator Protein-1 (AP-1), that regulates gene expression, and it can be related to uncontrolled cell growth, survival, and migration, all of which are hallmarks of cancer. These signaling processes lead to an increase in APP transcription and subsequent Aβ production, potentially influencing the pathogenesis of AD (Figure 6).[37]

Moreover, emerging evidence suggests that the *in vivo*-lysosomal pathway plays a regulatory role in the pathophysiology of AD, as alterations in this pathway can contribute to the accumulation of harmful proteins.[38] A research study conducted by PENG *et al.* investigated the impact of APOE4 expression on brain exosomes, utilizing human *post-mortem* tissue and mouse models humanized for apolipoprotein E.[39] These findings revealed that APOE4 carriers had lower levels of exosomes in the brain extracellular space, when compared to APOE3, and this reduction is associated with a downregulation of exosome biosynthesis and release, which may hinder the efficient removal of neurotoxic proteins, specifically Aβ*,* contributing to the development of neurodegenerative disorders, such as AD.[39]

In healthy brains, Aβ can be bound by the Sortilin-Related receptor (SORL1) and sent to the lysosomes for destruction. However, a study by ZOLLO *et al.* found that SORL1 expression was reduced, and Aβ levels were elevated in neural stem cells from patients who carried two APOE4 alleles.[40] As SORL1 plays a crucial role in Aβ clearance by guaranteeing its correct degradation in lysosomes, its reduction can disturb this essential process, leading to impaired Aβ degradation and an increase in this neurotoxic protein.[40]

According to TACHIBANA *et al.*, APOE4 can increase the production of Aβ by accelerating the process of APP endocytosis transport, which is controlled by LRP.[41] Low-Density Lipoprotein Receptor-Related Protein 1 (LRP1), a member of LRP family, contributes significantly to Aβ clearance, so pericytes, cells associated with the BBB, clear Aβ aggregates via LRP1/APOE interactions. However, these interactions are affected by APOE4 presence.[42]

These observations suggest that APOE4 may regulate Aβ accumulation in specific brain regions and may therefore play a crucial role in the pathogenesis of AD.

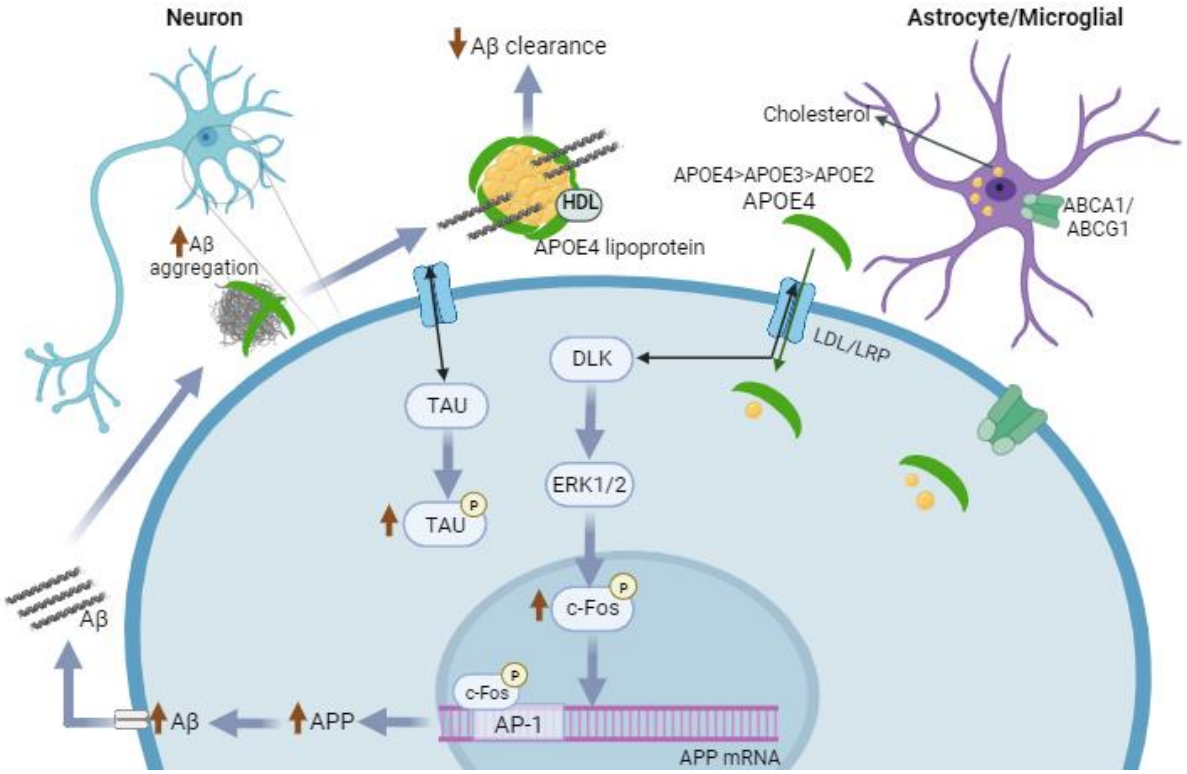

Figure 6. APOE4 effects in Aβ pathology. Glial-secreted APOE induces neuronal Aβ production in an order of potency of APOE4 > APOE3 > APOE2. When APOE4 binds to Aβ, a complex is formed that affects Aβ clearance and promotes its formation and deposition in the brain. APOE4 increases the APOE receptor's affinity for the DLK, activates ERK1/2 MAP kinase and causes cFos phosphorylation, promotes transcription factor AP-1, and as a consequence, increases APP transcription, improves APP expression, and subsequently elevates Aβ levels. DLK: Dual leucine-zipper kinase; ERK1/2: Extracellular Signal-Regulated Kinase 1 and 2; c-Fos: FBJ osteosarcoma; AP-1: Activator Protein-1; APP: Amyloid precursor protein; Aβ: Beta-amyloid; APOE: Apolipoprotein E; APOE2: Apolipoprotein E2; APOE3: Apolipoprotein E3; APOE4: Apolipoprotein E4; LDL: Low-Density Lipoprotein; LRP: Low-Density Lipoprotein Receptor-Related Protein; ABCA1: ATP-Binding Cassette Transporter A1; ABCG1: ATP-Binding Cassette Transporter G1.

5.1.3 ApoE4 and Tau aggregation

Tau protein promotes microtubule formation through tubulin polymerization and preserves its stability; however, when it is hyperphosphorylated, tau protein loses its biological function and leads to several detrimental effects.[43]

JABLONSKI *et al.* examined the role of three human APOE isoforms, specifically when produced by astrocytes, and concluded that APOE4 converts neuronal Tau to a more pathological state, a phenomenon not observed with the other APOE isoforms (Figure 6).[44]

Furthermore, HUANG *et al.* discovered that APOE4 is important in Tau pathology using three-dimensional human cerebral organoid (hCO) cells. The study revealed that both astrocytic and neuronal APOE4 increase Tau phosphorylation, a process associated with the abnormal alteration of Tau protein, and that may reduce neuron astrocyte transport of p-Tau, exacerbating Tau pathology.[45]

BENSON *et. al.* looked at the relationship between CSF biomarkers and APOE4 allele frequency in individuals with mild cognitive impairment (MCI), which includes AD. It confirms that APOE4 carriers higher Tau and p-Tau levels in a gene dosage dependent manner, which means that individuals with one APOE4 allele exhibited intermediate levels of these biomarkers in comparison to non-carriers and those with two APOE4 alleles.[4]

COOPER *et al.* demonstrated the involvement of LRP1 in Tau processing and its possible role in the pathogenesis of AD. LRP1 participates in the internalization and trafficking of Tau protein and efficiently transports Tau to lysosomes for degradation and this interaction is crucial for the proper clearance of Tau protein from the brain. However, the researchers revealed that phosphorylated forms of Tau, which refer to Tau protein that has endured abnormal phosphate group additions, bind weakly to LRP1 and are internalized less efficiently. This study also found that APOE4 inhibits Tau uptake via LRP-1-mediated transport, which results in defective Tau clearance and may contribute to the accumulation of pathogenic forms of Tau.[46]

6. Statins

6.1 Context and action mechanism

According to the World Health Organization, statins are the treatment of choice for hypercholesterolemia and associated cardiovascular disease, the leading global cause of morbidity and mortality. Statins are among the most prescribed medications worldwide, with an estimated 25 % of the world's population aged 65 and older currently receiving treatment

with statins, and the numbers are rising.[47, 48, 49] They inhibit the synthesis of cholesterol in the liver[49], by inhibiting HMG-CoA reductase, a membrane protein in the endoplasmic reticulum that catalyses the rate-limiting step in cholesterol biosynthesis, the acetylation of HMG-CoA to CoA and MVA (Figure 7).[50]

Numerous clinical trials demonstrate the efficacy of this class of drugs. Increasing evidence suggests that many of these clinical benefits may be attributable to mechanisms other than the cholesterol-lowering effect. Included among these so-called pleiotropic effects are anti-inflammatory, antioxidant, immunomodulatory, and anti-proliferative properties. These cholesterol-independent properties of statins prompted additional research into the treatment of various diseases. One of the identified mechanisms beyond statin pleiotropy is changes in membrane-associated proteins, whose function may change because of statin-induced modifications in protein prenylation or changes in lipid bilayer properties. Lipophilicity, elimination half-life, and potency vary among statins. Lipophilic statins such as simvastatin and lovastatin predominantly traverse cell membranes via passive diffusion, whereas pravastatin and rosuvastatin require carrier-mediated transport.[50]

There are currently three generations of HMGCR inhibitors on the market: the first generation consists of lovastatin, pravastatin, and fluvastatin; the second generation consists of simvastatin and atorvastatin; and the third generation consists of rosuvastatin and pitavastatin.[48] Statins, such as lovastatin and simvastatin, are associated with a decreased risk of AD.[51]

Recent data indicate that 27.9% of adults aged 40 or older are on statin therapy worldwide. In 2012–2013, 39.2 million people used statins, which resulted in 221 million prescriptions and $16.9 billion in U.S. sales. Several studies indicate a correlation between statin use and AD. Current AD meta-analyses suggest that statin use is associated with neurodegenerative disease (NDD) risk reductions, but additional research is required to contribute to the existing body of knowledge.[48]

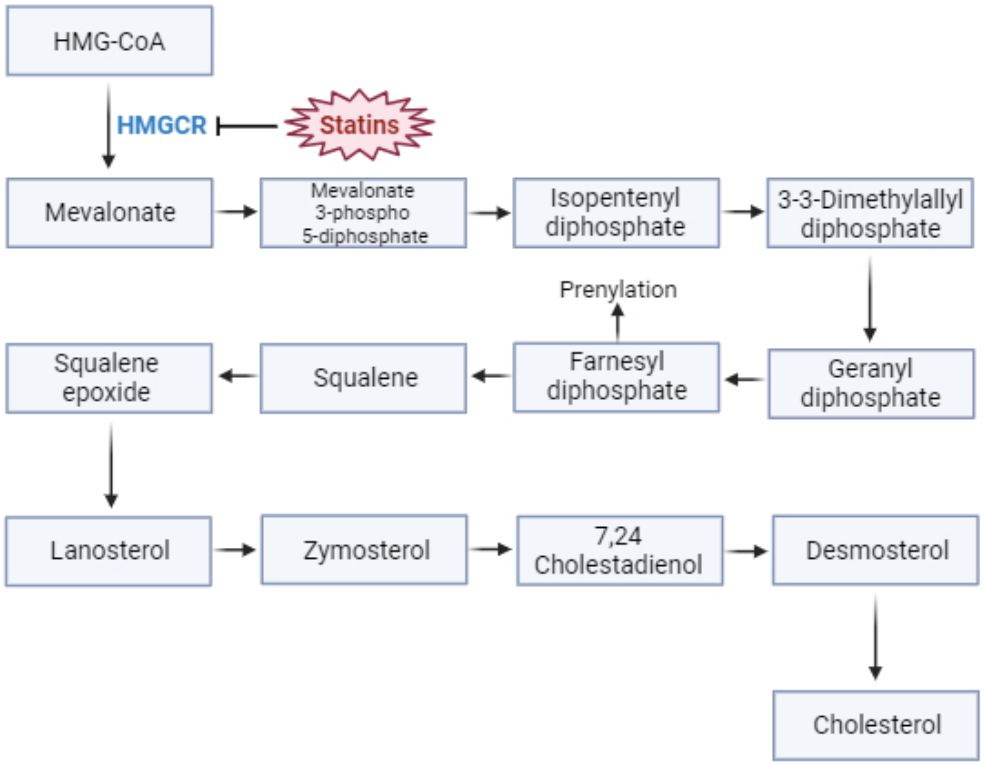

Figure 7: Simplified cholesterol biosynthesis pathway and the action of statins. Statins function by blocking cholesterol synthesis at the HMGCR's highly conserved catalytic region.

6.2 Paradoxal effects in Alzheimer's disease

6.2.1 Potential positive effects

Prospective epidemiological studies indicate that hyperlipidemia in midlife is a risk factor for late-onset AD, and *post-mortem* and *in vivo* imaging have identified associations between elevated LDL-C and increased cerebral Aβ load. Statins and other medications that reduce LDL-C have been proposed as potential AD treatments.[47] It has been reported that statins reduce the plasma concentration of esterified oxysterol in patients with hypercholesterolemia and elevated oxysterol levels.[52]

Whether or not statins affect cognitive function is a subject of considerable debate at present. Studies have provided evidence for both parties and reported both positive and negative effects. Overall, findings reported in the literature are exceedingly inconsistent. [47] Large randomized controlled trials (RCTs) could be helpful in clarifying the effects of dyslipidemia treatments on AD incidence without confounding, but such evidence is limited, and the slowly evolving pathogenesis of AD (at least a decade) makes it unsuitable as an

endpoint in trials of lipid-lowering drugs with relatively short intervention and follow-up periods (typically 2–5 years).[53]

In addition, independent studies have confirmed that statins influence cognitive function, reducing the risk of dementia in old age (by 25–50%) and the risk of AD. Certain combinations of an individual's gender and APOE genotype may limit the extent of these potential effects. Although statin use was analysed based on the presence or absence of a genetic risk factor for AD, APOE4, it seems the presence of ApoE4 did not influence the effects (or lack thereof) of statin use.[54]

In addition, statins exert numerous other effects, such as suppression of inflammatory responses, protection of neurons from excitotoxins, apoptosis, and oxidative stress, and promotion of synaptogenesis, all of which may be independent of their cholesterol synthesis inhibition. However, as mentioned before, the protection offered by statins against AD remains controversial.[55]

In the following section we summarize studies investigating the effect of statins on the underlying mechanisms associated with AD.

Study 1 - "Cholesterol-lowering drugs reduce APP processing to Aβ by inducing APP dimerization"

Statins are effective in reducing Aβ in neurons in control subjects without dementia as well as subjects with FAD and SAD. Statins inhibit cholesterol biosynthesis, and epidemiology studies have shown that statin users are at a lower risk for AD by altering APP processing and decreasing Aβ levels, as the β C-Terminal Fragment (βCTF) region of APP contains a transmembrane domain that can bind cholesterol.[56]

It has been proposed that statins inhibit the conversion of APP to Aβ. Statins decrease APP interaction with β-secretase (BACEI), modify APP dimerization, resulting in decreased APP, and promote dimerization of full-length APP (flAPP), which decreases processing into Aβ42 and Aβ40. Additionally, the increased dimerization of flAPP, resulting from statins action, interferes with BACE1 (the enzyme involved in cleaving APP) and APP interaction, leading to a reduction in Aβ production. Additionally, statins influence the turnover and metabolism of APP C-terminal fragments (CTF) (Figure 8).[56]

The MVA pathway for cholesterol biosynthetic regulates APP processing, and changes in cholesterol homeostasis can affect the production of Aβ. In particular, the researchers used a squalene synthase inhibitor and discovered that this also affected the processing of APP into

Aβ, corroborating the role of the MVA pathway. Both statins and squalene synthase inhibitors have been shown to decrease cholesterol esters and Aβ levels in this manner.[56]

As demonstrated by the reduction of Aβ in both wild-type and MAPT knockout neurons, statins can also reduce Aβ levels through a mechanism that is independent of Tau. This indicates that the levels of Aβ and pTau can be co-regulated by distinct, independent pathways involving variations in cholesterol metabolism.[56]

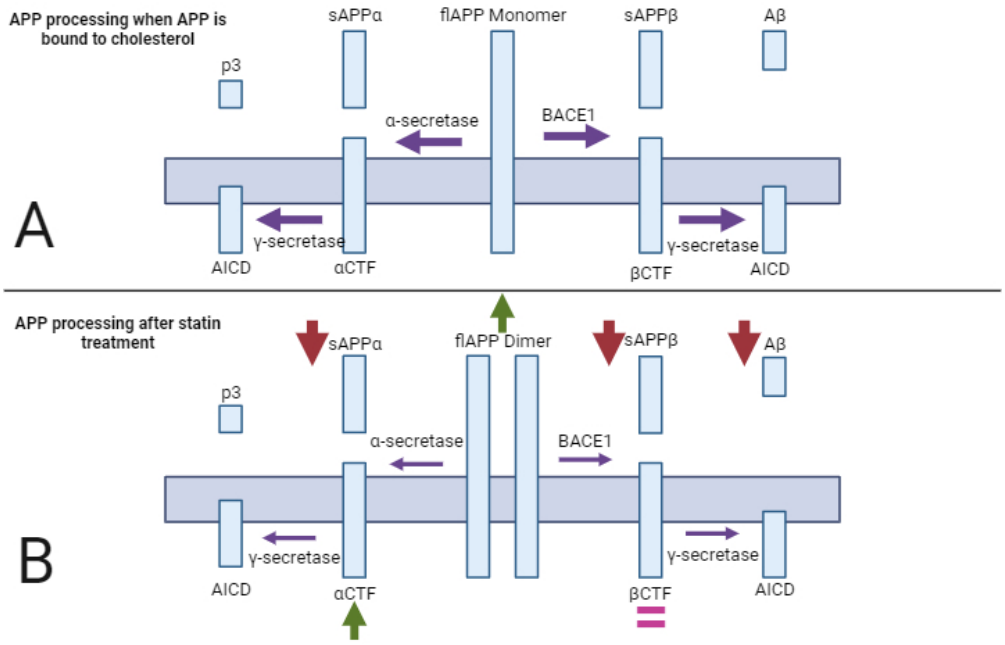

Figura 8. Cholesterol/CE-lowering drugs prevent APP processing by enhancing flAPP dimerization. (A) APP processing in untreated cells. (B) APP processing alterations identified after statin treatment. Statin treatment induces flAPP dimerization and accumulation, which inhibits APP processing, reduced sAPPα and sAPPβ, decreased APP-BACE1 interaction, and unmodified α-CTF and β-CTF levels, indicating statins also regulate CTF turnover. α-CTF: α-C-terminal fragments; β -CTF: β -C-terminal fragments; sAPP: Soluble amyloid precursor protein α; sAPPβ: Soluble amyloid precursor protein β; flAPP: Full-length amyloid precursor protein; BACE1: Beta-secretase 1; p3: Peptide 3; AICD: APP intracellular domain; APP: Amyloid precursor protein; Aβ: Beta amyloid. (Adapted from [56]).

<u>Study 2 - In silico evidence of direct interaction between statins and β-amyloid</u>

The molecular interaction between statins and Aβ peptides was investigated in this study. Using computer modelling, the researchers determined the affinity of different statins for Aβ based on the number and combinations of hydrogen bonds formed with the active site of Aβ. They discovered that pitavastatin, atorvastatin, rosuvastatin and fluvastatin had a higher affinity for Aβ than pravastatin, lovastatin, and simvastatin, compared with the standard ligand sulindac.[57]

The affinity of specific statins was correlated with the number and combinations of hydrogen bonds with the active site of Aβ but not with the target amino acid residues. These findings result in three hypothetical scenarios. Firstly, a direct molecular interaction between statins and Aβ protofibrils may underlie a direct statin-mediated effect on Aβ, which may be inhibitive. Second, the varying affinity between individual statins and Aβ protein could influence the variable anti-amyloidogenic potential of various molecules. However, this hypothesis does not appear to be supported by the available literature. Thirdly, further in vivo investigations are required to confirm the present findings in terms of determining the statins' affinity for Aβ.[57]

Nonetheless, the study provides evidence for a potential direct interaction between statin molecules and Aβ peptides, supporting previous research that identifies statins as antiamyloidogenic drugs and possibly paving the way for the development of novel AD disease-modifying therapies.[57]

Study 3 - The Cognitive Effects of Statins are Modified by Age

This investigation reported that the effect of statins on cognitive function varies with age and duration of treatment. The study examined cognitive performance in terms of reaction time, working memory, and fluid intelligence at baseline and at two follow-up evaluations within 5 to 10 years. It was found that the use of statins had a positive impact on reaction time in elderly individuals and fluid intelligence in both age groups, but a negative impact on working memory in younger participants.[47]

This study highlights the importance of characterizing modifiers of statin effects, such as age and duration of treatment, in order to obtain knowledge and formulate guidelines for prescribing statins and evaluating their side effects in patients. This is especially essential for midlife statin users, who may require a careful clinical evaluation. Overall, the study suggests that the effect of statins on cognitive function is complex and influenced by multiple variables, and that additional research is required to fully comprehend this effect.[47]

Study 4 - Simvastatin Efficiently Reduces Levels of Alzheimer's Amyloid β in Yeast

In study 4, simvastatin was shown to reduce the levels of the cellular Aβ42 protein dose-dependently and effectively. *Scharomicidae cervicae,* a basic eukaryotic model organism, demonstrated this. Compared to the other two statins, atorvastatin and lovastatin, simvastatin resulted in the greatest reduction. A comparison with fluconazole, which targets the same pathway of ergosterol synthesis, suggests that effects on ergosterol synthesis are not responsible for the decrease in Amyloid Beta 42-Green Fluorescent Protein (Aβ42-GFP).

Simvastatin effectively decreased levels of native Aβ42 in the population, providing new insights into how simvastatin exerts its neuroprotective function. This work hypothesizes that this decrease could be a result of protein clearance.[49]

Concerning the action mechanism of simvastatin, several investigations have examined the effects of statins on Aβ42 production. As depicted in Figure 4, HMGCR inhibition influences additional pathways, including protein prenylation. One of the proteases responsible for the production of Aβ42 from APP, BACE, is a prenylated protein. Numerous statins inhibit BACE prenylation, resulting in decreased Aβ42 production, as demonstrated by cultured mammalian cells.[49]

Simvastatin may inhibit Aβ42 production in the brain. Aβ42 is produced independently of protein prenylation, and protein clearance is hypothesized to be a significant factor in the current study. It is anticipated that the clearance of Aβ42 by statins will reduce the risk of AD by inducing a protein clearance response in cells with accumulated Aβ toxic proteins.[49]

Simvastatin is better when compared to other statins, such as atorvastatin and lovastatin, in its ability to prevent dementia. Furthermore, simvastatin was more effective than atorvastatin and lovastatin in eliminating Aβ42 fused to GFP. Simvastatin may also be more protective in humans because it is the most lipophilic statin, allowing for increased brain penetration.[49]

<u>Study 5 - Lipid-core nanocapsules containing simvastatin improve the cognitive impairment induced by obesity and hypercholesterolemia in adult rats</u>

Contrary to most other statins, a portion of simvastatin in circulation can cross the BBB. Although the use of statins in AD remains controversial, several *in vivo* studies have demonstrated the beneficial effects of simvastatin treatment on amyloidosis and Aβ plaque deposition. In addition, recent research has demonstrated that simvastatin improves cerebrovascular integrity by acting specifically at the BBB.[58] However, the study 5 mentions the difficulty of simvastatin and lovastatin entering the brain. Besides being metabolized by Cytochrome P450 (CYP), statins such as simvastatin and lovastatin may also be subject to efflux from P-glycoprotein (P-gp) systems that reduce their concentration and action in the tissues. Due to this, they have lower BBB penetration, which further diminishes their ability to treat diseases of the CNS.[51]

To address this issue, this investigation employed Polymer Nanocapsules (NCs). NCs have been studied as a drug delivery system that reduces side effects and enables drugs to cross the BBB, achieving a higher concentration of drugs in the CNS. In this study, researchers

developed and characterized the Simvastatin-Lipid Core Nanocapsules (SV-LNC) and Lovastatin- Lipid Core Nanocapsules (LV-LNC) structures and evaluated the structure with the highest statin burden, SV-LNC, in an animal model of obesity, hypercholesterolemia, and cognitive impairment typical of AD. Histological evidence of brain lesions in some of the animals fed with the high-fat diet supported the development of obesity, hypercholesterolemia, and cognitive impairment in animals fed with a hyperlipidic diet. It was observed that free simvastatin could reduce the enzymatic activity of Pyruvate Kinase (PK), a key enzyme for brain energy homeostasis, without altering the memory of animals fed with a standard diet. However, it had no effect on the cognitive impairment caused by a high-cholesterol and saturated-fat diet. Cognitive impairment is improved, however, when simvastatin is "concealed" in Lipid-Core Nanocapsules (LNC). Therefore, it is certain that SV-LNC is a promising alternative remedy for cognitive impairment.[51]

Study 6 - Statins and Brain Health: Alzheimer's Disease and Cerebrovascular Disease Biomarkers in Older Adults

The study 6 found that older adults on long-term statin therapy exhibited no differences (adverse or protective) in AD neuroimaging biomarkers compared to older adults not receiving statin therapy. Long-term statin exposure was, however, associated with diminished white matter integrity in the genu of the *corpus callosum*, which is consistent with a greater burden of cerebrovascular disease risk factors in this group.[59]

In addition, the potential relationship between circulating lipid levels and brain lipid metabolism was explored, as well as the heterogeneity of medication effects in research on aging and dementia. The authors propose a unifying hypothesis that chronic statin use in mid- to late-life does not appear to influence neuroimaging biomarkers of typical AD but may have the potential to influence brain structure and function and the resulting risk of dementia by altering cerebrovascular health.[59]

6.2.2 Uncertainties

The use of statins as therapeutic agents for dementia is highly controversial, with much scepticism regarding their efficacy.[60] As mentioned before, some investigations have suggested that statins may reduce the risk of dementia, AD, and cognitive impairment in certain patients. However, recent observational studies have concluded that statins are not associated with cognitive decline. Several randomized clinical trials with statins have failed to reduce the levels of AD biomarkers in the cerebrospinal fluid of asymptomatic patients or delay the progression of cognitive impairment in patients with mild to moderate AD symptoms[57] - although, case

studies have discovered cognitive and memory impairment in statin-treated patients, which highlights the persistent uncertainty surrounding the neurocognitive effects of statins.[61] This inconsistency among studies may be due to differences in the timing of statin therapy initiation or the severity of AD.[62]

Some statins can cross the BBB and reduce cholesterol levels in the brain. Since plasma lipoproteins are prevented from crossing the BBB, cholesterol levels in the brain are regulated autonomously. Cholesterol has been shown to play a significant role in the plasticity and synaptic function of the brain. Although, the reduction of brain cholesterol levels by statins may therefore inhibit brain function.[60]

As CHEN *et al.* mention[22], "aged brain needs a greater demand for cholesterol due to age-related cholesterol loss". So, reducing brain cholesterol levels could possibly have a negative impact on its correct functioning by increasing the risk of AD.[22] There is a growing disagreement regarding the role of cerebral cholesterol in AD. Therefore, the development of novel, more selective targets is required.[60]

7. Conclusions and Remarks

This disseration aimed to explore the complex ground of AD, addressing its pathophysiology and the crucial role that hereditary and environmental factors play in its development. The APOE4 gene variant stood out among these as a significant risk factor, greatly affecting susceptibility to AD. The thorough investigation of cholesterol metabolism and its complex interaction with AD highlighted the importance of preserving cholesterol homeostasis for preventing disease progression.

The study of statins, widely prescribed as cholesterol-lowering drugs, revealed their potential significance in modifying the risk of AD.

As previously stated, the effects of statins on AD continue to be controversial, with contradictory findings reported in the scientific literature. Some studies suggest that statins may have a protective role against AD, whereas others have found no significant association or even a possible adverse effect. In order to determine the potential benefits and hazards of statins in the prevention and treatment of AD, additional research is required.

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