UNIVERSIDADE FEDERAL DE MINAS GERAIS
FACULDADE DE FARMÁCIA

DÉBORA FERNANDES RODRIGUES

IMUNOSSUPRESSÃO E OBESIDADE NO PÓS-TRANSPLANTE HEPÁTICO

Belo Horizonte, MG
2018

DÉBORA FERNANDES RODRIGUES

IMUNOSSUPRESSÃO E OBESIDADE NO PÓS-TRANSPLANTE HEPÁTICO

Tese apresentada ao Programa de Pós-Graduação em Ciência de Alimentos da Faculdade de Farmácia da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do grau de Doutora em Ciência de Alimentos.

Orientadora: Prof[a] Dr[a] Adaliene Versiani Matos Ferreira

Coorientadora: Prof[a] Dr[a] Simone de Vasconcelos Generoso

Belo Horizonte, MG
2018

Rodrigues, Débora Fernandes.
R696i Imunossupressão e obesidade no pós-transplante hepático / Débora Fernandes Rodrigues. – 2018.
78f. : il.

Orientadora: Adaliene Versiani Matos Ferreira.
Coorientadora: Simone de Vasconcelos Generoso.

Tese (doutorado) - Universidade Federal de Minas Gerais, Faculdade de Farmácia, Programa de Pós-Graduação em Ciência de Alimentos.

1. Fígado – Transplante – Teses. 2. Obesidade – Teses. 3. Imunossupressão – Teses. 4. Metabolismo – Distúrbios – Teses. 5. Perda de peso – Teses. I. Ferreira, Adaliene Versiani Matos. II. Generoso, Simone de Vasconcelos. III. Universidade Federal de Minas Gerais. Faculdade de Farmácia. IV. Título.

CDD: 612.39

Elaborado por Aline Guimarães Amorim – CRB-6/2292

	UNIVERSIDADE FEDERAL DE MINAS GERAIS **PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIA DE ALIMENTOS**	**PPGCA**

FOLHA DE APROVAÇÃO

IMUNOSSUPRESSÃO E OBESIDADE NO PÓS-TRANSPLANTE HEPÁTICO

DÉBORA FERNANDES RODRIGUES

Tese submetida à Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em CIÊNCIA DE ALIMENTOS, como requisito para obtenção do grau de Doutor em CIÊNCIA DE ALIMENTOS, área de concentração CIÊNCIA DE ALIMENTOS.

Aprovada em 12 de novembro de 2018, pela banca constituída pelos membros:

Profa. Dra. Adaliene Versiani Ferreira Matos (Orientadora e Presidente da Comissão)
Escola de Enfermagem da UFMG

Profa. Dra. Simone de Vasconcelos Generoso
Escola de Enfermagem da UFMG

Profa. Dra. Helen Hermana Miranda Hermsdorff
Universidade Federal de Viçosa

Profa Dra. Andrezza Fernanda Santiago
Universidade Federal de Lavras

Profa. Dra. Helem de Sena Ribeiro
Instituto de Ensino e Pesquisa Santa Casa de BH

Profa. Dra. Karina Braga Gomes Borges
Faculdade de Farmácia da UFMG

Belo Horizonte, 12 de novembro de 2018.

Dedico este trabalho aos meus pais, Dimas Lelo Rodrigues e Lourdes Fernandes Rodrigues, aos meus irmãos, Gabriela Fernandes Rodrigues e Felipe Fernandes Rodrigues, e ao meu marido, Eduardo De Conti Teixeira Costa.

AGRADECIMENTOS

Expressar aqui minha gratidão a tudo e a todos será uma tarefa um tanto quanto difícil. Porém, com as palavras, tentarei expresar o que sinto nesse momento.

Primeiramente, meu agradecimento vai a **Deus**, que, nesses anos de intensa dedicação, tem me direcionado e aberto minha mente às diferentes oportunidades de crescimento que a vida oferece.

Agradeço aos meus pais, **Dimas Lelo Rodrigues** e **Lourdes Fernandes Rodrigues**, pela educação que me deram, pelo apoio incondicional em todas as etapas da minha vida e pelo amor que dedicam a mim todos os dias. A presença de vocês é e sempre será fonte de conforto e a expressão do amor para mim.

Aos meus irmãos, **Gabriela F. Rodrigues** e **Felipe F. Rodrigues**, pela convivência, paciência e carinho. Sei que conviver com uma doutoranda não é, muitas vezes, fácil devido aos compromissos e dedicação. O simples fato de ter vocês como irmãos já é suficiente para ter mais forças para seguir em frente. À minha vozinha que tanto amo, **Elza Alves de Andrade**, pelas constantes bênçãos e pelo carinho eterno. Aos meus demais **familiares** queridos e sempre presentes.

Ao meu marido, **Eduardo De Conti**, pela infinita paciência, pelos conselhos e por me tirar da frente do computador nos momentos em que isso era necessário. Você pode nem imaginar, mas sua presença foi essencial para aliviar o peso dos meus compromissos nesses anos todos. Obrigada por sua dedicação, amor e presença indispensável!

À minha sogra, **Ana Regina De Conti**, por torcer tanto por mim e por me alegrar com sua presença tão amável.

À minha querida orientadora, **Adaliene Ferreira**, por ser um exemplo para mim desde 2008, quando entrou na sala de aula feito uma jovem aluna, porém com uma habilidade de ensinar muito inspiradora. Sou grata a você por ter me acolhido de forma sempre carinhosa, alegre e sincera; por clarear as inúmeras dúvidas que, quando apresentadas a você, pareciam tão simples; por me ensinar coisas da vida, alheias à minha formação acadêmica, que talvez você nem saiba que me ensinou. É com imensa satisfação que sou sua aluna há 10 anos! Talvez você queira se livrar de mim (“desmamar” seus alunos, como você mesma diz), mas sinto dizer que me sinto tão parte do seu grupo que o considero, também, como meu eterno grupo!

À minha querida co-orientadora **Simone Generoso**, que me acompanha desde o mestrado. Fiquei muito feliz quando pude "te ter" como orientadora. Talvez você também não saiba, mas você me inspirou desde o dia em que nos conhecemos, quando eu fui sua estagiária docente. Você sempre me ensinou coisas do mundo acadêmico e de fora dele. Obrigada por ser tão alegre e companheira e por me ensinar sua visão de pesquisa e do ensino.

À professora **Isabel Correia**, que não mediu esforços em possibilitar a execução deste estudo, em fornecer equipamentos e o seu conhecimento tão vasto e enriquecedor.

Aos membros da banca de qualificação, professores **Lucilene Anastácio**, **Marina Oliveira** e **Cristiano Xavier**, pela imensa colaboração na interpretação dos dados. Aos membros da **banca de defese de tese**, professoras **Andrezza Santiago**, **Helem de Sena**, **Helen Hermana**, **Karina Braga**, **Letícia Cordeiro** e **Marina Oliveira**, pela disponibilidade e pela colaboração na finalização deste trabalho.

Às minhas colegas de coleta, que se tornaram grandes amigas: **Gabriela Fagundes**, **Nayara Monteze** e **Brenda Monteiro**. Espero que tenham MUITA consciência de que, sem vocês, esse trabalho não aconteceria! OBRIGADA por me ajudarem em todas as etapas desse estudo e por terem se tornado tão especiais em minha vida.

À minha "mommy" **Ana Maria Rodrigues**, por me permitir dividir as angústias desse trabalho e de outros, por me auxiliar nas análises estatísticas e por ter se tornado minha querida amiga.

Às minhas amigas do grupo: **Débora Romualdo**, por ser tão parceira, me incentivar a crescer na vida profissional e ser um exemplo para minha vida acadêmica; **Kátia Costa** e **Josiana Sabino**, por tantas vezes termos tido a oportunidade de dividir bancada, trocar confidências e por termos nos tornado amigas; **Marina Oliveira**, **Laís Bhering, Jaqueline Lana** e **Ana Letícia Malheiros**, por termos embarcado juntas nessa viagem acadêmica. A amizade de vocês fez tudo mais fácil e agradável. Muito do que sei hoje aprendemos juntas ou aprendi com vocês. Obrigada! **Marina Zicker** e **Cíntia Tarabal**, pela convivência tão agradável e pelas colaborações que fizemos nesses anos.

À **Érica Vieira**, pela importante colaboração e dedicação na realização dos diversos experimentos deste trabalho. Ao professor **Antônio Lúcio Teixeira**, pelo auxílio na execução destes experimentos.

Às minhas queridas eternas amigas, **Ana Paula Della Nina**, **Geraldina Zwaal** e **Luciana Tertuliano**, pela amizade e apoio desde 2007!

Às minhas colegas e amigas, professoras da Faculdade Ciências da Vida, **Maria Góes**, **Fernanda Amaral**, **Edina Rodrigues**, **Silvia Fernandes e Hélem Ribeiro**, pelas caronas tão divertidas, pelo apoio na finalização dessa etapa e por serem grandes exemplos para mim.

Às minhas super-nutris de Betim, **Lívia Marcossi**, **Tatiane de Oliveira** e **Renata Lisboa**, por fazerem minha vida tão mais leve e feliz. Tenho certeza de que criamos uma grande amizade desde 2015!

Às colegas da Faculdade São Camilo, em especial à **Adriana Graciano**, pelo convívio tão agradável e amizade.

Ao **Mauro Ramalho Silva**, querido amigo que me orientou durante a Iniciação Científica e me incentivou a seguir a carreira acadêmica.

Ao professor **Mauro Martins Teixeira**, pela imensa colaboração na minha vida acadêmica desde o mestrado. Sou muito grata a você e aos demais membros do grupo de pesquisa Imunofar!

Aos **voluntários** desse trabalho, peças essenciais para a execução desse estudo. Expresso a todos vocês a minha gratidão!

Aos **funcionários do Laboratório do Hospital das Clínicas**, que nos auxiliaram nas coletas de sangue.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (**CAPES**) pelo apoio financeiro. À **FAPEMIG**, à **pro-reitoria de pesquisa** da UFMG e ao **Programa de Pós-graduação em Ciência de Alimentos**-FAFAR/UFMG, pelo apoio na execução desse trabalho.

Por fim, sou muito grata a essa etapa da minha vida que, hoje, se finaliza de certa forma. Obrigada a todos que passaram por minha vida nessa etapa e a tudo o que vivi nesses anos todos!

“Descobrir consiste em olhar para o que todo mundo está vendo e pensar uma coisa diferente”.

Roger Von Oech

SUMÁRIO

RESUMO

Com o atual crescimento do número de casos de sobrepeso e obesidade no mundo, é crescente a demanda por estudos que busquem a compreensão dos elementos envolvidos no ganho de peso corporal. Nesse sentido, tem sido demonstrado que a obesidade está em estreita associação com a inflamação crônica subclínica, evidenciando o papel fisiológico da resposta inflamatória na obesidade. De fato, trabalhos têm demonstrado que pacientes imunossuprimidos apresentam ganho de peso excessivo, como ocorre em indivíduos transplantados hepáticos. Assim, o objetivo deste trabalho foi investigar se o comprometimento da resposta inflamatória de transplantados de fígado afeta o controle do peso corporal. Para isso, pacientes foram pareados com indivíduos controles não-transplantados. Ambos os grupos foram acompanhados por 6 meses. Nesse período, os voluntários seguiram um plano alimentar para perda de peso e foram acompanhados para verificação de mudanças no peso e na composição corporal, adesão dietética, ingestão alimentar, taxa metabólica de repouso, frequência de atividade física e para coleta de sangue. Os transplantados de fígado apresentaram taxa metabólica de repouso menor que os indivíduos controles, o que pode ser um fator de risco importante para o ganho de peso excessivo no pós-transplante hepático. Pacientes transplantados apresentaram, ainda, perfil semelhante de células mononucleares periféricas em relação aos controles, com destaque apenas para as células NK $CD8^{low}CD56^{+}CD16^{+}$ e linfócitos B, que apresentaram menor e maior frequência, respectivamente, no grupo transplante. Além disso, esse grupo apresentou menores concentrações séricas de citocinas, como IFN-γ, TNF, IL-4, IL-2 e IL-10, e menor responsividade inflamatória das células mononucleares periféricas sob diferentes estímulos inflamatórios. Após 3 e 6 meses de acompanhamento, respectivamente, o grupo controle perdeu em média 1,77% e 3,73% de peso, diferente do grupo transplantado, que perdeu apenas 0,42% e 0,54% do peso corporal, respectivamente. No grupo controle houve, ainda, melhora da composição corporal, com diminuição da massa gorda e da circunferência de cintura, e manutenção da massa magra, o que não foi observado no grupo transplantado. Assim, este trabalho traz evidências de que pacientes transplantados hepáticos são hipometabólicos e que a inflamação pode exercer papel importante como mediadora do processo de perda de peso.

Palavras-chave: Transplante hepático. Obesidade. Imunossupressão. Hipometabolismo. Perda de peso. Intervenção dietética.

ABSTRACT

With the current worldwide growth in the number of cases of overweight and obesity, there is a demand for studies to understand the elements involved in the gaining of body weight. It has been demonstrated that obesity is in close association with chronic subclinical inflammation, evidencing the physiological role of the inflammatory response in obesity. In fact, studies have shown that immunosuppressed patients show excessive weight gain, as occurs in liver recipients. Thus, the objective of this study was to investigate whether the compromised inflammatory response of liver recipients affects body weight control. For this, recipients were paired with non-transplanted controls. Both groups were studied for 6 months. During this period, volunteers followed a weight loss dietary planning and were followed to check changes in body weight and composition, dietary adherence and intake, resting energy expenditure, frequency of physical activity, and blood collection. Liver recipients showed lower resting energy expenditure than controls, which may be an important risk factor for excessive weight gain in post-liver transplantation. Transplanted patients also had a similar profile of peripheral blood mononuclear cells when compared to controls, highlighting NK CD8lowCD56 + CD16 + and B lymphocytes, which showed lower and higher frequency, respectively, in the transplant group. In addition, this group had lower serum concentrations of cytokines, such as IFN-γ, TNF, IL-4, IL-2 and IL-10, and lower inflammatory responsiveness of peripheral blood mononuclear cells under different inflammatory stimuli. After 3 and 6 months of follow up, respectively, the control group lost in average 1.77% and 3.73% of weight, different from the liver transplanted group, which lost only 0.42% and 0.54% of body weight, respectively. In the control group, there was also an improvement in body composition, with decreased fat mass and waist circumference, and lean mass maintenance, which was not observed in the transplanted group. Thus, this work provides evidence that liver recipients are hypometabolic and that inflammation may play an important role in the weight loss process.

Keywords: Liver transplantation. Obesity. Immunosuppression. Hypometabolism. Weight loss. Dietary intervention.

1. INTRODUÇÃO

A obesidade é definida como o acúmulo excessivo de gordura corporal (WHO, 2016), que pode culminar no desenvolvimento de outras doenças crônicas não-transmissíveis, como diabetes, hipertensão (BABU *et al.*, 2018), doenças cardiovasculares (KOTSIS *et al.*, 2018), câncer (ALI *et al.*, 2018; LIN *et al.*, 2018), dentre outras. Dados da Organização Mundial da Saúde (WHO, 2016) apontam que cerca de 39% e 13% da população mundial apresenta sobrepeso e obesidade, respectivamente. No Brasil, a última pesquisa do Ministério da Saúde (BRASIL, 2017) mostrou que, no conjunto de 27 cidades brasileiras avaliadas, o excesso de peso atinge 53,8% da população, sendo 18,9% das pessoas consideradas obesas.

Com o atual aumento no número de casos de sobrepeso e obesidade no mundo, é crescente a demanda por estudos que busquem a compreensão dos elementos envolvidos no ganho de peso corporal. Nesse sentido, tem sido demonstrado que a obesidade está em estreita associação com a inflamação crônica subclínica, apontada como elo entre o excesso de peso e as comorbidades associadas (NDISANG *et al.*, 2014). Um passo importante na compreensão da associação entre o tecido adiposo e o sistema imunológico se deu em 1993, quando Hotamisligil, Shargill e Spiegelman (1993) mostraram, pela primeira vez, aumento da expressão do Fator de Necrose Tumoral (TNF) em adipócitos de animais obesos. Posteriormente, foi evidenciado que indivíduos obesos apresentam maiores concentrações plasmáticas de diversas citocinas (WEGHUBER *et al.*, 2014; SINDHU *et al.*, 2015), ressaltando que a obesidade é, de fato, caracterizada por processo inflamatório crônico de baixa intensidade.

Neste contexto, trabalhos têm sido realizados com o intuito de aprimorar a compreensão acerca do papel do sistema imunológico no desenvolvimento da obesidade. Hoje, sabe-se que o tecido adiposo é composto não somente por adipócitos, mas por conjunto diverso de células, como fibroblastos, pre-adipócitos, células endoteliais e imunológicas (RINK *et al.*, 1996; DUFFAUT *et al.*, 2009). Além disso, esse tecido secreta inúmeras citocinas que agem de forma autócrina, parácrina e endócrina, regulando o metabolismo (GREENBERG e OBIN, 2006). À medida em que esse tecido se expande, ocorrem modificações a nível molecular e estrutural, acompanhadas por alterações na resposta inflamatória (KOSTELI *et al.*, 2010; ASTERHOLM *et al.*, 2014).

Dessa forma, é claro na literatura que a obesidade está em estreita associação com a inflamação. No entanto, não se sabe até que ponto essa resposta inflamatória é patológica ou mesmo fisiológica. Essa última é uma questão recentemente discutida e que encontra embasamento, principalmente, em trabalhos experimentais, os quais têm demonstrado que a inefetividade da resposta inflamatória está atrelada ao rompimento do controle da estocagem ou da mobilização de gordura em camundongos (KOSTELI *et al.*, 2010; MENEZES-GARCIA *et al.*, 2014; MARTINS *et al.*, 2018). Apesar desses dados não terem sido demonstradaos em humanos, ensaios clínicos têm evidenciado que o uso de imunossupressores pode levar a excessivo ganho de peso e de gordura corporal (SARACENO *et al.*, 2008; RENZO *et al.*, 2011; BROWN *et al.*, 2012; SONG *et al.*, 2014). De fato, pacientes em uso de imunossupressores, como aqueles que realizaram transplante de órgãos, apresentam ganho de peso expressivo após a cirurgia (BIANCHI *et al.*, 2008; ANASTACIO *et al.*, 2013; KUGLER *et al.*, 2015; LOPEZ-VILELLA *et al.*, 2015). Anastácio et al. (2012) mostraram que a maior parte do peso perdido durante a espera pelo transplante de fígado, por exemplo, é recuperada durante o primeiro ano pós-operatório. Esses autores mostraram que, após 3 anos da realização da cirurgia, os pacientes ganharam, em média, 11,6 kg, sendo que 56,4% deles estavam acima do peso. Richards et al. (2005) observaram, também, que 67% dos pacientes apresentaram sobrepeso ou obesidade 3 anos após a realização do transplante de fígado.

O ganho de peso após o transplante hepático pode ser decorrente de vários fatores, como uso de drogas imunossupressoras (CHARLTON *et al.*, 2017), balanço energético positivo (RIBEIRO *et al.*, 2014), etiologia da doença hepática (ANASTACIO *et al.*, 2012), sedentarismo (KUGLER *et al.*, 2015) e desenvolvimento de estado hipometabólico (RICHARDSON *et al.*, 2001). Entretanto, as causas para o ganho de peso corporal após o transplante ainda não são claras e, até o momento, estudos não foram realizados com o objetivo de associar o estado imunossuprimido como um fator importante no controle da homeostase metabólica e da perda de peso corporal. Dessa forma, a hipótese deste trabalho é a de que a inibição da resposta inflamatória, mediante uso de medicamento imunossupressor, altera o controle do peso corporal em indivíduos transplantados de fígado. Para isso, este trabalho foi dividido em dois capítulos com os seguintes objetivos: (I) investigar se ocorre alteração do gasto energético de repouso após o transplante hepático; (II) caracterizar o perfil imunológico de pacientes imunossuprimidos após transplante de fígado e avaliar se eles respondem a uma intervenção nutricional para perda de peso.

2. REVISÃO DE LITERATURA

2.1. Obesidade e inflamação

Nos últimos anos, diversos trabalhos foram realizados na tentativa de compreender os fatores de risco e de agravo ao desenvolvimento da obesidade e doenças associadas. Desde então, tem se tornado clara a associação entre a resposta inflamatória e as doenças metabólicas, incluindo obesidade, resistência à insulina, dislipidemia, etc (RANA *et al.*, 2007). Classicamente, a inflamação é definida como a resposta principal do organismo para lidar com lesões ou infecções, cujas características incluem inchaço, vermelhidão, dor e febre (LARSEN e HENSON, 1983). Essa resposta a curto prazo é essencial para restaurar a homeostase e promover o reparo tecidual. Uma vez que o agente causador da resposta inflamatória é removido ou neutralizado, a inflamação é resolvida. No entanto, a resposta inflamatória associada à obesidade apresenta-se com características distintas da inflamação clássica, sendo caracterizada como crônica e de baixo grau, ou mesmo como "metainflamação" (HOTAMISLIGIL, 2006), uma referência à inflamação desencadeada por uma disfunção metabólica.

Sabe-se que os nutrientes são importantes gatilhos para o desencadeamento da resposta inflamatória na obesidade (LOPEZ-GARCIA *et al.*, 2005; AEBERLI *et al.*, 2011; RODRIGUES *et al.*, 2014). O grupo de pesquisa do presente trabalho (Imunometabolismo) e outros têm reiterado esse fato, uma vez que alguns estudos mostraram que nutrientes estimulam resposta inflamatória tanto sistêmica quanto localizada em diferentes tecidos, como o adiposo e o hepático (KOSTELI *et al.*, 2010; RODRIGUES *et al.*, 2014). Trabalho anterior do presente grupo de pesquisa mostrou que alterações metabólicas e inflamatórias ocorrem tão cedo quanto no estado pós-prandial (RODRIGUES *et al.*, 2014). Neste estudo, camundongos alimentados com carboidratos refinados, como frutose e sacarose, apresentaram alterações metabólicas e inflamatórias a nível sistêmico e localizado no tecido adiposo e no fígado até quatro horas após o consumo (RODRIGUES *et al.*, 2014). De forma semelhante, outro trabalho do mesmo grupo avaliou se a resposta inflamatória também é exacerbada em humanos sadios após consumo de frutose (RODRIGUES *et al.*, dados não publicados). Foi observado que a ingestão aguda desse carboidrato, associado a uma refeição

padronizada, levou à hiperlipemia e aumento da quantidade de leucócitos circulantes no período pós-prandial.

Tais trabalhos mostraram a resposta aguda frente a diferentes nutrientes. No entanto, à medida em que esse tipo de hábito alimentar obesogênico se mantém, as respostas metabólica e inflamatória se tornam crônicas e desencadeam as disfunções associadas à obesidade. O trabalho de Lopez-Garcia (2005) demonstrou que o consumo crônico de gorduras trans aumenta as concentrações plasmáticas de marcadores inflamatórios e de disfunção endotelial. De forma semelhante, Aeberli et al. (2011) estudaram os efeitos do consumo crônico de bebidas adoçadas com diferentes acúcares e observaram alterações em marcadores de risco cardiovascular e inflamatórios, como aumento das concentrações sanguíneas de LDL, glicose, proteína C reativa e da relação cintura-quadril.

Muito embora sejam frequentes os trabalhos que associam o consumo excessivo de determinados nutrientes à resposta inflamatória, ainda não é claro o papel do sistema imunológico no controle da adiposidade. Conforme mencionado acima, os trabalhos comumente apontam o papel patológico da inflamação na obesidade, associando-a com o desenvolvimento de outras doenças crônicas. De fato, muitas vezes são evidenciados apenas os aspectos patológicos da inflamação, e há pouco entendimento acerca do propósito fisiológico que ela pode exercer na obesidade. Com relação a isso, tem sido proposto conceito de “inflamação fisiológica” ou “para-inflamação”, que, segundo Medzhitov (2008), seria uma resposta adaptativa com características intermediárias entre o estado basal e o inflamatório. Segundo o mesmo autor, a resposta para-inflamatória não é acionada por lesão ou infecção no tecido, mas sim pelo seu mau funcionamento, a fim de restaurar sua funcionalidade e homeostase. Caso essa disfunção tecidual perdure, por exemplo, por meio do consumo excessivo de certos nutrientes, a para-inflamação se torna crônica e deletéria, com o desenvolvimento de outras desordens associadas ao ganho de peso. O que este e outros autores propõem é que a inflamação exerce papel fisiológico na manutenção da homeostase tecidual, monitorando o mau funcionamento do tecido e promovendo sua adaptação a condições adversas e estados disfuncionais (MEDZHITOV, 2008; RODRIGUES *et al.*, 2014; CHOE *et al.*, 2016).

Para garantir a homeostase tecidual, o tecido adiposo abriga outros tipos celulares que não somente adipócitos maduros armazenando uma grande gota lipídica. Tal tecido é composto por várias células estromais, incluindo pré-adipócitos, células

endoteliais, fibroblastos e células imunológicas (RINK *et al.*, 1996; DUFFAUT *et al.*, 2009). No armazenamento crônico e excessivo de energia que caracteriza a obesidade, a expansão do tecido adiposo promove acúmulo anormal de células imunológicas nesse tecido, especialmente macrófagos, para controlar o alto fluxo de lipídeos e o estresse tecidual (KOSTELI *et al.*, 2010). Além de aumentarem em número, os macrófagos também são alterados fenotipicamente durante a obesidade: enquanto o tecido adiposo saudável abriga predominantemente macrófagos M2, com características anti-inflamatórias, o tecido de obesos contém macrófagos M1, pró-inflamatórios (CHYLIKOVA *et al.*, 2018). Estes últimos são importantes fontes de citocinas pró-inflamatórias, como TNF e IL-6, que podem bloquear a ação da insulina nos adipócitos e sistemicamente (HOTAMISLIGIL *et al.*, 1993; KUO *et al.*, 2017; LI *et al.*, 2017). Além disso, outros mediadores inflamatórios encontram-se alterados na obesidade, como adiponectina, leptina, visfatina, resistina, IL-1β, IL-10, dentre outros (OUCHI *et al.*, 2011; MAKKI *et al.*, 2013). Tais mediadores exercem funções fisiológicas importantes que podem se alterar com a ruptura da homeostase do tecido adiposo. Como exemplo, pode-se citar o aumento da lipólise (ZHANG *et al.*, 2002), desregulação do apetite e do balanço energético (ROSENBAUM e LEIBEL, 2014), alteração da sensibilidade à insulina (HOTAMISLIGIL *et al.*, 1993; AHLSTROM *et al.*, 2017; KUO *et al.*, 2017; LI *et al.*, 2017), disfunção endotelial (ZHANG, 2008) e alteração na produção de fatores angiogênicos (CORVERA e GEALEKMAN, 2014). Assim, a regulação da função e da plasticidade do tecido adiposo é alterada na obesidade, comprometendo o funcionamento deste e de outros tecidos, como muscular, hepático e endotelial (VIRDIS, 2016; WU e BALLANTYNE, 2017; SARWAR *et al.*, 2018).

Para entender, portanto, o papel do sistema imunológico na obesidade e a contribuição da resposta inflamatória para a homeostase do tecido adiposo, o grupo de pesquisa Imunometabolismo tem realizado seus estudos, principalmente, em modelos animais. Tais trabalhos permitiram o embasamento científico que direcionou, em parte, a construção da hipótese deste trabalho. Inicialmente, Menezes-Garcia et al. (2014) estudaram o efeito de uma dieta indutora de obesidade em camundongos com baixa responsividade inflamatória após diferentes estímulos. Tais camundongos, com deleção genética do receptor do fator de ativação plaquetária, apresentaram maior adiposidade, embora menos inflamação no tecido adiposo, após consumo crônico de dieta indutora de obesidade, quando comparados com o grupo selvagem que recebeu a mesma dieta.

Dessa forma, os autores sugeriram que a inflamação presente nos animais obesos poderia estar envolvida no controle da expansão do tecido adiposo. De forma semelhante, outro trabalho do grupo de pesquisa mostrou que camundongos com deleção genética do receptor do TNF apresentaram baixas concentrações de citocinas no tecido adiposo, o que foi associado à expansão desse tecido após ingestão de dieta padrão (MARTINS *et al.*, 2017). Mais recentemente, Lacerda et al. (dados não publicados) avaliaram o papel da inflamação no remodelamento do tecido adiposo de camundongos após 24 horas de jejum. Os autores observaram que, após o jejum, houve aumento de citocinas e do recrutamento de células imunológicas para o tecido adiposo, o que é corroborado por outros autores (KOSTELI *et al.*, 2010).

Estes trabalhos demonstram, em suma, que o milieu inflamatório é importante para o controle da expansão e da redução do tecido adiposo e ratificam, portanto, a hipótese já apontada na literatura de que a inflamação tem um propósito fisiológico no controle da adiposidade (MEDZHITOV, 2008; GREGOR e HOTAMISLIGIL, 2011). Por outro lado, trabalhos que envolvem seres humanos na tentativa de reforçar essa hipótese são escassos. Alguns ensaios clínicos têm evidenciado que o uso de drogas imunossupressoras está atrelado ao ganho de peso. Parmentier-Decrucq et al. (2009) acompanharam pacientes com doença de Chron em uso de medicamento bloqueador da ação do TNF e observaram aumento de cerca de 18% na deposição de gordura abdominal. Outro estudo comparou os efeitos dos imunossupressores azatioprina e betametasona em pacientes com dermatite e observaram que houve ganho de peso em ambos os grupos, sendo que o o último levou a maior ganho ponderal (VERMA *et al.*, 2008). Assim, este trabalho se propõe a atrelar as evidências experimentais de que a baixa responsividade inflamatória pode interferir no controle da adiposidade em camundongos com os ensaios clínicos que salientam o ganho de peso em pacientes em uso de terapia imunossupressora.

2.2 Ganho de peso no pós-transplante de órgãos sólidos

Tem sido relatado na literatura que muitos receptores de órgãos, como rins, coração, pulmão e fígado, apresentam ganho de peso excessivo após o transplante (BIANCHI *et al.*, 2008; KUGLER *et al.*, 2015). De fato, a prevalência de excesso de

peso nessa população chega a valores maiores que os observados na população em geral (RICHARDS, J. *et al.*, 2005; FUSSNER *et al.*, 2015; LOPEZ-VILELLA *et al.*, 2015). O trabalho de Fussner et al. (FUSSNER *et al.*, 2015) demonstrou que cerca de 40% dos pacientes transplantados hepáticos se tornaram obesos dentro de 3 anos. Outro trabalho relatou que cerca de 80% dos receptores de fígado apresentam algum grau de obesidade abdominal até 4 anos após o transplante (ANASTACIO *et al.*, 2011). Já outros autores demonstraram que esses pacientes ganham aproximadamente 5 kg no primeiro ano pós-transplante, podendo alcançar um ganho de 10 kg até o terceiro ano, com aproximadamente 30% se tornando obesos (RICHARDS, *et al.*, 2005). De forma semelhante, López-Vilella (2015) relatou que 72% dos pacientes transplantados cardíacos apresentaram obesidade 1 ano após a cirurgia.

O ganho de peso no pós-transplante é, muitas vezes, interpretado como um fator positivo na recuperação da saúde desses pacientes (SCHUTZ *et al.*, 2012). No entanto, sabe-se que o ganho de peso excessivo leva a complicações importantes, como hipertensão (BULUM *et al.*, 2015), doenças cardiovasculares (LAURES *et al.*, 2005; FUSSNER *et al.*, 2015), diabetes (ANDRADE *et al.*, 2017; ABDULRAHMAN *et al.*, 2018) e dislipidemia (RIBEIRO *et al.*, 2014), sendo considerado fator de risco para a rejeição do enxerto e, até mesmo, a morte (CONZEN *et al.*, 2015). Já foi demonstrado que o ganho de peso excessivo no primeiro ano de transplante renal, representando aumento de 5% no índice de massa corporal (IMC) dos pacientes, aumenta as chances de perda do enxerto (DUCLOUX *et al.*, 2005). Além disso, o ganho de peso maior que 20% e 10% no primeiro e no segundo anos pós-transplante, respectivamente, foram associados à morte (CHANG e MCDONALD, 2008).

Tendo em vista a alta prevalência de obesidade e sobrepeso em pacientes transplantados com diferentes órgãos e as complicações ocasionadas pelo peso excessivo, trabalhos têm sido conduzidos com o objetivo de entender as causas para esse ganho de peso após a cirurgia. Especificamente em relação ao transplante hepático, estudos têm apontado diferentes causas, como balanço energético positivo (FERREIRA *et al.*, 2013; RIBEIRO *et al.*, 2014), etiologia da doença hepática (ANASTACIO *et al.*, 2012), sedentarismo e hábitos alimentares inadequados, particularmente no primeiro ano pós-transplante (RICHARDS *et al.*, 2005; KUGLER *et al.*, 2015), hipometabolismo (RICHARDSON *et al.*, 2001) e terapia imunossupressora (NEAL *et al.*, 2001; ROGERS *et al.*, 2005). No entanto, ainda não há consenso sobre as reais causas para o ganho de peso excessivo no pós-transplante hepático.

Dentre as hipóteses levantadas acima, destaca-se o estado hipometabólico e o uso de drogas imunossupressoras. Com relação à primeira, os trabalhos têm sido contraditórios, uma vez que alguns confirmam (RICHARDSON *et al.*, 2001; FERREIRA *et al.*, 2013) e outros negam (PERSEGHIN *et al.*, 2002; RIBEIRO *et al.*, 2014) essa hipótese, gerando a necessidade de mais estudos. Tais trabalhos são contraditórios por utilizarem métodos diferentes para avaliação do gasto energético – como por meio da comparação do gasto energético aferido por calorimetria indireta com o estimado por equações preditivas (RIBEIRO *et al.*, 2014; CHEN *et al.*, 2016) – ou por avaliarem pacientes em diferentes períodos após o transplante de fígado (FERREIRA *et al.*, 2013; CHEN *et al.*, 2016). Essa discussão também é levantada em outros tipos de transplantes. Heng et al. (2015) estudaram pacientes transplantados renais em uso de terapia imunossupressora à base de inibidores de calcineurina, sem o uso de corticosteroides. Aqueles que haviam ganhado peso apresentaram menor gasto energético em relação àqueles com peso normal. Acredita-se, portanto, que a compreensão do estado metabólico desses pacientes permitirá a criação de melhores estratégias com foco na individualização do plano alimentar, na tentativa de evitar o desenvolvimento de obesidade e suas complicações.

Com relação à terapia imunossupressora, os trabalhos também têm sido controversos, uma vez que muitos demonstram que o ganho de peso excessivo no pós-transplante hepático se deve ao uso de corticosteroides (ROGERS *et al.*, 2005). De fato, tais drogas podem aumentar o apetite e alterar o metabolismo energético (DALLMAN *et al.*, 2004). No entanto, os pacientes continuam ganhando peso após a retirada de tais medicamentos (RICHARDS *et al.*, 2005). O trabalho de Richards et al. (2005) demonstrou que não houve diferença no ganho de peso de receptores de fígado que foram ou não tratados com corticosteroides por mais ou menos que 3 meses. Ainda no mesmo trabalho, os autores avaliaram possíveis diferenças no ganho de peso de pacientes tratados com inibidores de calcineurina, como tacrolimus e ciclosporina, agentes imunomoduladores empregados usualmente no pós-transplante hepático. Os autores mostraram que aqueles tratados com ciclosporina apresentaram maior ganho de peso no primeiro ano pós-transplante, mas que, por volta de 2 anos, o ganho de peso foi similar entre aqueles tratados com ciclosporina ou tacrolimus. De forma semelhante, outro trabalho verificou que o uso de ciclosporina levou a maior ganho de peso em pacientes transplantados cardíacos, quando comparado ao uso de tacrolimus (LOPEZ-VILELLA *et al.*, 2015). Apesar de a terapia imunossupressora ser considerada como um

possível fator na indução do ganho excessivo de peso corporal após o transplante hepático, os mecanismos atrelados a isso ainda não foram esclarecidos. Ademais, não há trabalhos que realizaram intervenção nutricional para perda de peso em receptores de fígado após o primeiro ano da realização do transplante, período em que há ganho expressivo de peso corporal (RICHARDS *et al.*, 2005; ANASTACIO *et al.*, 2012) e adaptação ao novo estilo de vida.

Tanto a ciclosporina quanto o tacrolimus são drogas imunossupressoras que revolucionaram a realização de transplantes em todo o mundo desde a sua descoberta, sendo considerados como tratamento padrão para pacientes transplantados (AZZI *et al.*, 2013). Em indivíduos saudáveis, a ativação de células T eleva as concentrações intracelulares de cálcio, o que ativa a calcineurina intracelular, levando à desfosforilação do fator de transcrição NFAT (do inglês "nuclear factor of activated T cells"). Tal fator ativa a transcrição de genes que codificam diferentes citocinas e moléculas co-estimulatórias, como IL-2, IL-4 e CD40 (BRAM *et al.*, 1993; SHIBASAKI *et al.*, 1996; RAO *et al.*, 1997; AZZI *et al.*, 2013). A produção de IL-2, particularmente, estimula o crescimento e a diferenciação de células T (SHIBASAKI *et al.*, 1996). Assim, a ciclosporina e o tacrolimus inibem a atividade fosfatase da calcineurina e, consequentemente, a ativação de células T, tornando-os essenciais na indução da tolerância ao enxerto pós-transplante (AZZI *et al.*, 2013). Além disso, já foi demonstrado que o tacrolimus diminui a produção de múltiplas citocinas, como IL-3, IL-4, IL-5, IL-13, IFN-γ, IL-12, IL-11, IL-18, TGF-β, TNF, IL-1β e IL-6 (DUTTA e AHMAD, 2011; CARR, 2013; CHANG *et al.*, 2016). Dessa forma, como já foi descrito o papel do sistema imunológico no controle da homeostase metabólica e do peso corporal, o presente estudo se baseia em duas hipóteses: (I) pacientes transplantados hepáticos são hipometabólicos, o que configura um fator de risco para o ganho de peso excessivo; e (II) receptores de fígado não respondem efetivamente a uma intervenção nutricional para perda de peso devido ao comprometimento imunológico.

3. OBJETIVOS

3.1 Objetivo geral

Investigar fatores associados ao controle do peso corporal de pacientes transplantados hepáticos.

3.2 Objetivos específicos

- Investigar se pacientes transplantados hepáticos apresentam diminuição do gasto energético de repouso (Capítulo 1).

- Caracterizar o perfil imunológico de pacientes após transplante hepático (Capítulo 2).

- Elucidar se o estado imunossuprimido de transplantados de fígado interfere na efetividade da intervenção nutricional com foco na perda de peso (Capítulo 2).

4. CAPÍTULO 1: Hypometabolism as a potential risk factor for overweight and obesity in liver recipients

Artigo aceito para publicação na revista *Nutrition*, fator de impacto 3.734, Qualis A1 em Ciência de Alimentos.

Hypometabolism as a potential risk factor for overweight and obesity in liver recipients

Débora Fernandes Rodrigues[1], Nayara Mussi Monteze[1], Gabriela Barbosa Pires Fagundes[1], Brenda Loise Monteiro[1], Agnaldo Soares Lima[2], Maria Isabel Toulson Davisson Correia[2], Simone de Vasconcelos Generoso[1], Adaliene Versiani Matos Ferreira[1]

[1] Immunometabolism, Department of Nutrition; Nursing School; Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[2] Alpha Institute of Gastroenterology; Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Corresponding author: PhD Adaliene Versiani Matos Ferreira. E-mail: adaliene@gmail.com. Phone: +5531 34098036. Department of Nutrition, Nursing School, Universidade Federal de Minas Gerais. Av. Prof. Alfredo Balena, 190 - Santa Efigênia, Belo Horizonte, Minas Gerais, Brazil. Zip Code: 30130-100.

Abbreviations: BMI: body mass index; FFM: fat-free mass; LT: liver transplantation; METs: Metabolic Equivalents; REE: resting energy expenditure; WC: waist circumference.

ABSTRACT

Objective: The aim of the present study was to identify whether overweight liver recipients are hypometabolic.

Methods: LT recipients (n=20) who were 18-65 years old, had a body mass index (BMI) ≥ 25 kg/m^2 and had completed 1-3 years since the operation were matched with healthy controls concerning sex, age, BMI and body composition. Dietary intake data were collected using a 3-day food record. The subjects' daily activities were converted into Metabolic Equivalents. REE was assessed in the morning, after an overnight fast (12h), by indirect calorimetry, using an open-circuit calorimeter.

Results: The total energy and macronutrient intakes were similar among liver recipients and controls. The majority of the subjects from both groups were sedentary (75%; n=15/each group). Patients who underwent LT showed lower REE (1,449.15 ± 101.25 kcal) when compared to the control group (1,768.45 ± 86.94 kcal). Likewise, the REE/ fat-free mass (FFM) ratio was lower in the LT group (LT: 28.9 ± 1.7 kcal/ kg vs C: 32.9 ± 0.9 kcal/ kg of FFM; $P< 0.05$). The correlation between the FFM and the REE was strong in controls (r= 0.73; $P<0.01$), while it was moderate in the LT group (r= 0.45).

Conclusion: The REE of overweight liver recipients is reduced and it might be a risk factor for the excessive body weight gain in this population. This trial was registered at clinicaltrials.gov as NCT03103984.

Keywords: weight gain; liver transplantation; resting energy expenditure; tacrolimus; body mass index.

INTRODUCTION

Recent advances in the treatment of patients who underwent liver transplantation (LT) have resulted in a substantial increase in survival rates. Consequently, long-term complications such as obesity (1), hypertension (2, 3), diabetes (4), dyslipidemia (5), cardiovascular disease (2, 6) and other metabolic disorders have become prevalent. Additionally, excessive body weight gain after the operation is an important risk factor for graft failure and death (7), which highlights the utmost importance to adopt effective strategies to control the exacerbated adiposity.

Multiple factors have been implicated in the weight gain following LT, including the positive energy balance (8, 9), the etiology of liver disease (10) and the immunosuppressive regimen (11, 12). Physical inactivity and inadequate dietary habits are also factors that might affect body weight, particularly in the first year after surgery (13, 14). On the other hand, there is a hypothesis that these patients might be hypometabolic (15), but this has not been well elucidated yet and it is sometimes excluded (8).

The liver is an immunologically and metabolically privileged organ. It acts as a hub to connect various tissues, including the skeletal muscles, the adipose tissue, the gut and the brain (16). Multiple nutrients, hormones and neuronal signals regulate liver metabolism. Furthermore, immunosuppressive drugs are used to downregulate the immune system activity after LT in order to reduce the risk of organ rejection. Taking into account the notorious integration of metabolism and the immune system (17, 18), it is also important to consider that the immunosuppressed state might play a role in the weight gain following LT. Our hypothesis is that liver recipients develop a state of hypometabolism, which plays a key role in the weight gain in the long term after surgery.

There are limited data in the literature about resting energy expenditure (REE) in subjects who underwent LT. Only few studies regarding health conditions and the new lifestyle after LT were carried out in the long run when most of the patients are more stable. Moreover, the metabolic status of LT recipients is frequently evaluated based on predictive equations (8), which may lead to limited interpretations.

Herein, the aim of the present study was to identify whether overweight liver recipients are hypometabolic, which can be a possible factor associated to body weight

gain after LT. A better understanding about the metabolic status of liver recipients could help individualize dietary treatments after LT.

MATERIAL AND METHODS

Study design and participants

This was a cross-sectional study conducted as part of a baseline evaluation on studies of immunosuppression and obesity in transplanted patients. The study was carried out at Hospital das Clínicas/ Universidade Federal de Minas Gerais, Brazil, and was approved by the ethics committee of that university (CAAE: 30409114.8.0000.5149). The ClinicalTrials.gov identifier is NCT03103984. Between August 2014 and May 2016, we screened the data from LT recipients and selected those who were 18-65 years old, had a BMI ≥ 25 kg/m2 and 1-3 years after the operation. This post-transplantation period was chosen because it is known that it is when many of the liver recipients become overweight and have adapted themselves to the new routine (14). The exclusion criteria were: re-graft, pregnancy, breast-feeding, external nutritional counseling in progress and use of hormonal therapy or weight loss medication. Patients were then invited to participate in the study.

LT recipients were matched with non-transplanted controls concerning sex, age, BMI and body composition. Subjects from the control group followed the same inclusion/exclusion criteria applied to the LT group, except they were no liver transplant patients and were not in use of immunosuppressive or anti-inflammatory drugs. Bariatric surgery patients were also excluded.

The procedures regarding the study protocol were explained to subjects, who were required to give written consent.

Procedures

The participants were interviewed to assess their demographic and socioeconomic data, eating habits, physical activity levels and clinical parameters. To assess their anthropometric data, body weight and height were measured to the nearest 0.1 kg and 0.1 cm, respectively, using a floor model scale/stadiometer (Filizola□). Waist circumference were obtained using an inextensible measuring tape with accuracy of 0.1 cm and it was measured at the level of the umbilicus (19). Body composition was

determined by bioelectrical impedance analysis (Quantum X - RJL Systems, Inc., Clinton Township, Michigan). BMI was calculated and classified according to the World Health Organization (20).

Dietary intake data were collected using a 3-day food record. We also used pictures representing portion sizes and household measures to estimate food weights. We provided instructions to all volunteers concerning how to complete the record, i.e, fill it in with 3 non-consecutive days throughout the week they entered the study, considering 1 weekend day. Food intake data were entered into the software Brasil Nutri® and converted into calories, carbohydrates, proteins and lipids, using tables of food composition (21, 22).

Participants were asked about their routine in order to transform their daily activities into Metabolic Equivalents (METs) (23). The obtained values were then multiplied by the time spent on the respective activity, summed and divided by 24 (corresponding to one day). From this, we were able to classify individuals into sedentary (<1.4), limited activity (1.55-1.60) and physically active (≥ 1.75) (20).

REE was assessed in the morning, after an overnight fast (12h), by indirect calorimetry (IC), using an open-circuit calorimeter (MetaCheck™ metabolic rate analysis system, model 7100, Korr Medical Technologies) (24, 25). The calorimeter was auto-calibrated prior to each measurement according to the manufacturer's instructions. Subjects were placed in a quiet, temperature-controlled room (22-24 oC) and remained seated throughout the study. After 30 minutes of acclimatization, measurements were made over 10 minutes. They were instructed to relax, breathe normally and minimize movement. The REE was then divided by kilogram of fat-free mass (REE/FFM).

Statistical analysis

The sample size was estimated in 20 participants. For this, we considered a standard deviation of 226.7 kcal in the REE of liver recipients, based on a study carried out with a similar population [8]; a confidence interval of 95.0% with an alpha level of 0.05; and an acceptable error of 100.0 kcal.

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) software, version 19.0 (IBM Corporation, Armonk, NY, USA). For descriptive purposes, categorical variables were compared using the chi-square test. All data were

analyzed for normality using the Kolmogorov-Smirnov test. Comparisons between both groups were performed using Student's T or Mann-Whitney tests, for parametric and nonparametric variables, respectively. Correlation between REE and FFM were calculated with Pearson's coefficient, since these variables were parametric. Differences were considered statistically significant at the $P < 0.05$ level.

RESULTS

Out of 100 patients, eighty were excluded due to the following reasons: insulin use (n=7), re-graft (n=10), age less than 18 (n=4) or more than 65 years old (n=13), BMI< 25 kg/m2 (n=30), external nutritional counseling in progress (n=2) and lack of interest (n=14). Thus, a total of 20 patients met the inclusion criteria and accepted to be included in the study (**Figure 1**).

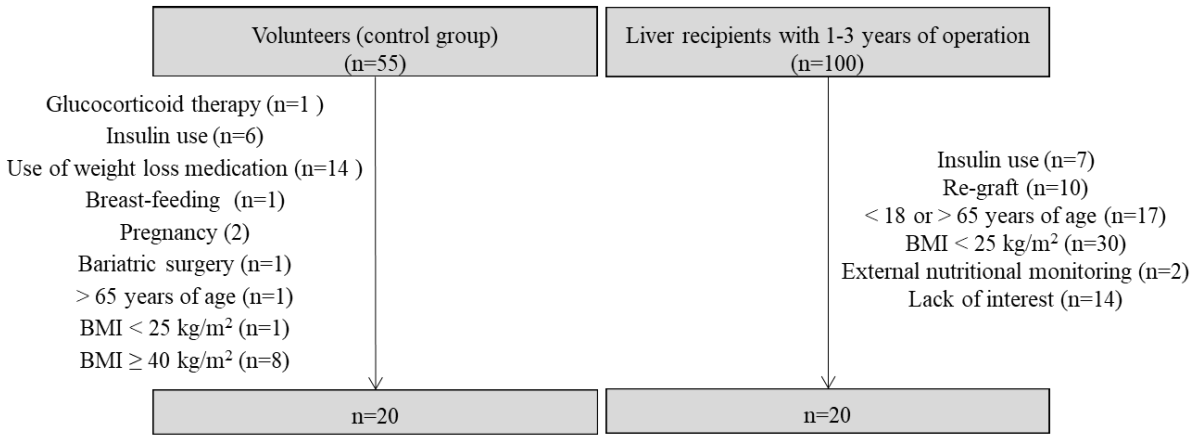

FIGURE 1. Flow diagram of the study.

Baseline characteristics of the patients and controls are listed in **Table 1**. Most of the LT patients were male (n=14; 70%) with a mean age of 50 ± 3 years old. There was no difference between the marital status and the family income in both groups. The mean BMI of the LT patients was 30.10 ± 0.89 kg/m² and they had, in average, 102.11 ± 2.09 cm of waist circumference, 35.99 ± 1.68% of body fat and 64.01 ± 1.68% of FFM. All characteristics listed above match those from the control group.

The average time since LT was 26 ± 2 months (**Table 1**). The most frequent indication for LT were alcohol use (40%; n=8) followed by hepatitis C virus infection (30%; n=6), cryptogenic cirrhosis (15%; n=3) and autoimmune hepatitis (15%; n=3).

Most of the patients (n=18, 90%) were treated with tacrolimus and only 2 (10%) were in use of cyclosporine. Two (10%) patients were also treated with corticosteroids (**Table 1**).

TABLE 1. Baseline characteristics of controls and patients who underwent liver transplantation

Variables	**Controls (n=20)**	**Liver transplantation (n=20)**	***P***
Sex [n (%)]			
Male	12 (60%)	14 (70%)	$P>0.05^{c}$
Female	8 (40%)	6 (30%)	
Age (mean ± SEM)	45 (± 2)	50 (± 3)	$P>0.05^{a}$
Marital Status [n (%)]			
Single	1 (5%)	2 (10%)	$P>0.05^{c}$
Married	17 (85%)	14 (70%)	
Divorced	2 (10%)	4 (20%)	
Family income [$ (min - max)]	953.35 (462.62-3,833.87)	740.26 (231.31-4,464.22)	$P>0.05^{b}$
BMI (kg/m²) (mean ± SEM	31.19 ± 0.58	30.10 ± 0.89	$P>0.05^{a}$
WC (cm) (mean ± SEM)	104,32 ± 1.69	102.11 ± 2.09	$P>0.05^{a}$
Body fat (%) (mean ± SEM)	35.78 ± 1.92	35.99 ± 1.68	$P>0.05^{a}$
Fat-free mass (%) (mean ± SEM)	64.22 ± 1.92	64.01 ± 1.68	$P>0.05^{a}$
Time since LT (months) (mean ± SEM)	-	26 ± 2	
Indication for LT [n (%)]:			
Alcohol abuse	-	8 (40%)	
Hepatitis C virus	-	6 (30%)	
Cryptogenic cirrhosis	-	3 (15%)	
Autoimmune hepatitis	-	3 (15%)	
Immunosuppressive treatment [n (%)]			
Tacrolimus	-	18 (90%)	
Cyclosporine	-	2 (10%)	
Corticosteroids	-	2 (10%)	

[a] Student's T-Test; [b] Mann-Whitney; [c] Chi-square. BMI: body mass index; LT: liver transplantation; WC: waist circumference.

The total energy and macronutrient intakes (**Table 2**) were not different among liver recipients and controls. There was no difference between the level of physical activity of both groups (**Table 3**) and the majority of them were classified as sedentary (75%; n=15/each group).

TABLE 2. Energy and macronutrient intake of controls and patients who underwent liver transplantation

Variables	**Controls (n=20)**	**Liver transplantation (n=20)**	***P***
Energy intake (kcal) (mean ± SEM)	1799.62 ± 151.73	1601.27 ± 294.85	*P*>0.05[a]
Carbohydrates (g) (mean ± SEM)	222.73 ± 13.17	204.62 ± 18.62	*P*>0.05[a]
Proteins (g) (mean ± SEM)	79.18 ± 5.99	83.11 ± 16.48	*P*>0.05[a]
Lipids (g) (mean ± SEM)	70.42 ± 5.43	66.49 ± 12.72	*P*>0.05[a]

[a] Student's T-Test

TABLE 3. Physical activity level of controls and patients who underwent liver transplantation

Physical activity level	**Controls (n=20)**	**Liver transplantation (n=20)**	***P***
Sedentary [n (%)]	15 (75%)	15 (75%)	
Limited activity [n (%)]	4 (20%)	1 (5%)	*P*>0.05[c]
Active [n (%)]	1 (5%)	4 (20%)	

[c] Chi-square

As mentioned, the subjects (cases and non-cases) included in this study showed the same body composition to avoid bias when comparing the REE of both groups. Patients who underwent LT showed lower REE (1,449.15 ± 101.25 kcal) when compared to the controls (1,768.45 ± 86.94 kcal) at the *P*=0.02 level (**Figure 2**). The REE/ FFM ratio was also lower in the LT group (LT: 28.9 ± 1.7 kcal/ kg vs C: 32.9 ± 0.9 kcal/ kg of FFM; *P*< 0.05).

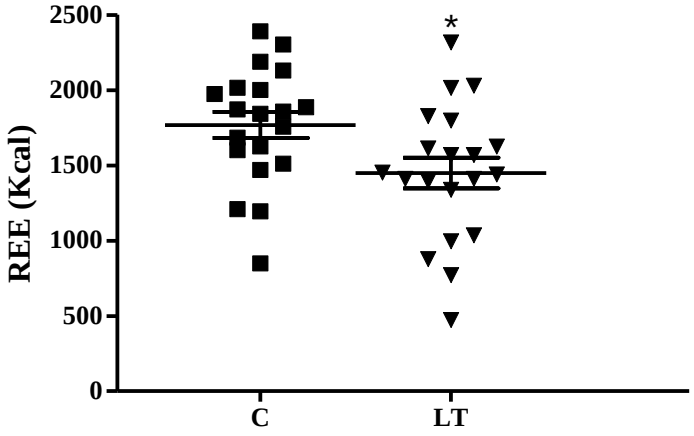

FIGURE 2. Resting energy expenditure (kcal) of controls (C) and patients who have undergone liver transplantation (LT). The values were measured by indirect calorimetry. Data are expressed as the means ± SEM; *P<0.05.

We found that the FFM was not strongly correlated with the REE in the LT group. Whilst this correlation was found to be strong in controls (r= 0.73; P<0.01), it was moderate in the LT group (r= 0.45) at the P=0.05 level.

DISCUSSION

Obesity is considered a multifactorial disease and is often a consequence of multiple factors, including sex, age, genetic factors, sedentary lifestyle, decline in REE, high energy intake, and social and cultural aspects (26-28). Among these numerous factors, we raised the hypothesis that the low resting energy expenditure could be a potential risk factor related to overweight after LT. Since the liver plays a central role in energy metabolism, it is reasonable to speculate that liver recipients have altered physiological responses that lead to an excessive body weight. Herein, we have showed that overweight liver recipients have low metabolic rates that may be related to an obese phenotype 1-3 years after the transplant.

Some authors have also measured the REE of patients after LT, but conflicting results have been the rule. Richardson et al. (15) measured the REE of liver recipients using IC and observed a reduction in the energy expenditure 9 months after hospital discharge. In a similar way, Ferreira et al (29) followed patients throughout the first year

after LT. The REE of these patients increased after 30 days and reduced in an average of 226 kcal at the end of the study period (one year after the transplant). These studies are important to reinforce the premise that LT downregulates the energy expenditure of the recipients. However, both studies addressed the individuals' metabolic rate comparing patients' data before and after transplant. We, in turn, provided a comparison between overweight liver recipients with non-transplanted paired controls, allowing us to better understand the metabolic changes and the development of obesity after LT.

A recent study evaluated changes in the metabolic rate of liver recipients during resting and exercise (30). The authors studied patients transplanted for nonalcoholic steatohepatitis (NASH) and compared them with nonalcoholic fatty liver disease (NAFLD) controls. In accordance with our data, they demonstrated that liver recipients, particularly females, have both lower resting and exercise energy expenditure compared to controls. Although this study reinforces our hypothesis, it was limited to patients transplanted for NASH. Herein, we evaluated liver recipients transplanted for different reasons, allowing a general conclusion and a broader application in the clinical practice.

Conversely, other studies have shown that post-transplant patients are not hypometabolic (8, 31). A previous study from our group (8) was not able to show the hypometabolism in LT subjects after 6.5 years of the operation. In a similar way, another study measured the REE of patients 6, 14 and 32 months after LT (31). The authors did not find any difference in the REE after LT. The discrepancies between these studies and ours are mainly owing to the different methodological approaches. First, in the above studies, the authors applied the Harris-Benedict (HB) formula (32) to classify patients as hypo-, normo- or hypermetabolic. Indeed, the metabolic status is frequently studied by comparing the REE with predictive equations (8, 33). The use of these formulas is more convenient because they are practical, inexpensive and may spare the use of controls. However, our current data show that these formulas may be not fully adequate when applied to liver recipients. This might be a consequence of the methods used to develop the HB equation, which was developed based on data collected from healthy volunteers (32). The HB equation has been shown to be imprecise in a diversity of clinical settings (34, 35). Second, the above studies did not establish a BMI inclusion criterion. The degree of overweight has been shown to be a significant factor influencing the accuracy of the predictive equations (36). Furthermore, this aspect might be of particular importance to detect the hypometabolism in liver recipients. We believe

that the comparison between the paired groups made it possible to show the hypometabolism in the liver recipients, undetected in other studies.

A factor that can influence energy expenditure is the dietary composition (37). It is known that it could affect energy expenditure directly, by differences in macronutrient composition, or indirectly, through hormonal responses to diet (37). Likewise, the practice of physical activity is known to impact the energy expenditure in a dose-dependent manner (38). We showed here that both control and LT groups had the same energy and macronutrient intake, as well as a similar level of physical activity, which may imply that both were not major determinants of the REE in these subjects. Other features can influence the energy expenditure, such as sex (39), age (40), BMI (41) and FFM (42). Herein, we carefully controlled the groups concerning all these variables to avoid bias.

Another important determinant of the REE in healthy subjects is the FFM, which accounts for approximately 70% of the variance in REE (42, 43). Surprisingly, we did not find a strong correlation between REE and FFM in liver recipients as we did in controls. When the energy expenditure was adjusted to the FFM, liver recipients showed lower values. Although not evaluated here, it can be explained, at least in part, by a decrease in the mitochondrial function. A previous study indirectly assessed the mitochondrial function of short-term liver recipients and controls using the 13C-labeled ketoisocaproic acid ([13C]KICA; 2-keto[1_13C]isocaproic acid) breath test as well as by measuring the REE through IC (44). For some liver recipients, the median REE was 17%–18% higher than controls, as showed by other studies evaluating short-term liver recipients (29, 45). On the other hand, the tacrolimus exposure was correlated with the lower REE and respiratory quotient. Other studies also showed that tacrolimus impairs the mitochondrial function (46, 47), but this topic needs to be better studied.

Other hypotheses can be postulated to explain why LT downregulates metabolism. First, we believe that the immunosuppression plays a key role in this phenomenon by the lack of inflammatory response. It was previously shown that inflammatory cytokines induce energy expenditure (17, 48), which led us to suppose that a low immunological competence may influence the energy expenditure. Second, it is possible that the transection of the hepatic nerves after LT (49) has led a metabolic disintegration. In fact, vagal sensory neurons innervate the liver, such as other visceral organs, providing a link between the central nervous system and the metabolic response to control feeding (50). Vijgen et al. (51) studied the relationship between vagus nerve

stimulation and energy expenditure in epileptic patients and found a positive relationship. Furthermore, vagal nerve stimulation can prompt the central nervous system to decrease eating and increase expression of neurotrophic factors able to stimulate energy expenditure (50). On the other hand, other studies have demonstrated hepatic reinnervation at certain time points after LT (52, 53). Thus, the metabolic implications of this denervation in transplanted livers remains to be determined.

The present study has some limitations. First, the patients were recruited from the same care center. However, based on the limited data about the topic, the restricted number of liver-transplanted volunteers and the satisfactory sample size, we believe that our data will help the development of adequate care strategies applied to liver recipients. Another limitation is that the 3-day food record, a tool applied to estimate energy and macronutrient consumption, is frequently a questionable method (54), mainly because the underreporting of dietary intake is common, notably in overweight individuals (55). On the other hand, it is currently the available tool and it has been extensively applied in different studies (8, 29) showing high validity (56) and providing a trustworthy estimation about the subjects' intake.

Overall, we believe that many factors contribute to body weight gain after LT. Nevertheless, our findings demonstrate that a reduced rate of energy expenditure is present in overweight liver recipients and, thus, it might be a risk factor for body weight gain. It is unclear, however, what causes the hypometabolic status, and studies are needed to clarify this.

DECLARATIONS OF INTEREST

None.

ACKNOWLEDGMENT

We thank our colleague Ana Maria dos Santos Rodrigues for the statistical analysis assistance.

Funding: this study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and Pró-Reitoria de Pesquisa da UFMG.

AUTHOR'S CONTRIBUTIONS

A.V.M.F. designed the research; D.F.R, N.M.M, G.B.P.F, B.L.M conducted research; D.F.R, M.I.T.D.C, S.V.G. and A.V.M.F analyzed data; D.F.R, M.I.T.D.C, S.V.G. and A.V.M.F wrote the paper; A.S.L. and M.I.T.D.C provided essential materials; A.V.M.F. had primary responsibility for the final content. All authors read and approved the final manuscript.

REFERENCES

1. Anastácio LR, Ferreira LG, de Sena Ribeiro H, Lima AS, Garcia Vilela E, Toulson Davisson Correia MI. Body Composition and Overweight of Liver Transplant Recipients. Transplantation. 2011;92(8):947-51.

2. Fussner LA, Heimbach JK, Fan C, et al. Cardiovascular disease after liver transplantation: When, What, and Who Is at Risk. Liver Transplantation. 2015;21(7):889-96.

3. Perito ER, Lustig RH, Rosenthal P. Metabolic Syndrome Components After Pediatric Liver Transplantation: Prevalence and the Impact of Obesity and Immunosuppression. American Journal of Transplantation. 2016;16(6):1909-16.

4. Andrade AR, Bittencourt PL, Codes L, et al. New Onset Diabetes and Non-Alcoholic Fatty Liver Disease after Liver Transplantation. Ann Hepatol. 2017 November-December;16(6):932-40.

5. Ribeiro HdS, Anastácio LR, Ferreira LG, Lagares ÉB, Lima AS, Correia MITD. Prevalence and factors associated with dyslipidemia after liver transplantation. Revista da Associação Médica Brasileira. 2014;60(4):365-72.

6. Mirabella S, Brunati A, Ricchiuti A, Pierini A, Franchello A, Salizzoni M. New-onset diabetes after liver transplantation. Transplant Proc. 2005 Jul-Aug;37(6):2636-7.

7. Conzen KD, Vachharajani N, Collins KM, et al. Morbid obesity in liver transplant recipients adversely affects longterm graft and patient survival in a single-institution analysis. HPB (Oxford). 2015 Mar;17(3):251-7.

8. Ribeiro HS, Anastácio LR, Ferreira LG, Lima AS, Correia MITD. Energy expenditure and balance among long term liver recipients. Clinical Nutrition. 2014;33(6):1147-52.

9. Ferreira LG, Santos LF, Anastacio LR, Lima AS, Correia MI. Resting energy expenditure, body composition, and dietary intake: a longitudinal study before and after liver transplantation. Transplantation. 2013 Sep;96(6):579-85.

10. Rezende Anastacio L, Garcia Ferreira L, Costa Liboredo J, et al. Overweight, obesity and weight gain up to three years after liver transplantation. Nutr Hosp. 2012 Jul-Aug;27(4):1351-6.

11. Rogers CC, Alloway RR, Buell JF, et al. Body weight alterations under early corticosteroid withdrawal and chronic corticosteroid therapy with modern immunosuppression. Transplantation. 2005 Jul 15;80(1):26-33.

12. Neal DA, Gimson AE, Gibbs P, Alexander GJ. Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids, and weight. Liver Transpl. 2001 Jun;7(6):533-9.
13. Kugler C, Einhorn I, Gottlieb J, et al. Postoperative weight gain during the first year after kidney, liver, heart, and lung transplant: a prospective study. Prog Transplant. 2015 Mar;25(1):49-55.

14. Richards J, Gunson B, Johnson J, Neuberger J. Weight gain and obesity after liver transplantation. Transplant International. 2005;18(4):461-6.

15. Richardson RA, Garden OJ, Davidson HI. Reduction in energy expenditure after liver transplantation. Nutrition. 2001 Jul-Aug;17(7-8):585-9.

16. Rui L. Energy metabolism in the liver. Compr Physiol. 2014 Jan;4(1):177-97.

17. Ye J, Keller JN. Regulation of energy metabolism by inflammation: a feedback response in obesity and calorie restriction. Aging (Albany NY). 2010 Jun;2(6):361-8.

18. Lackey DE, Olefsky JM. Regulation of metabolism by the innate immune system. Nat Rev Endocrinol. 2016 Jan;12(1):15-28.

19. WHO - World and Health Organization. Waist Circumference and Waist-Hip Ratio, Report of a WHO Expert Consultation2011 8–11 December 2008.

20. WHO - World and Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation. World Health Organ Tech Rep Ser. 2000;894:i-xii, 1-253.

21. USDA - U.S. Department of Agriculture. USDA Food Composition Databases. 2018.

22. NEPA. Tabela Brasileira de Composição de Alimentos – TACO 4ª edição revisada e ampliada. Campinas, SP. 2011:161.

23. Ainsworth BE, Haskell WL, Whitt MC, et al. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000 Sep;32(9 Suppl):S498-504.

24. Taghadomi Masoumi Z, Eshraghian MR, Hedayati M, Pishva H. Association between uncoupling protein 2, adiponectin and resting energy expenditure in obese women with normal and low resting energy expenditure. Gynecological Endocrinology. 2017:1-5.

25. Rodrigues AMS, Costa ABP, Campos DL, et al. Low validity of predictive equations for calculating resting energy expenditure in overweight and obese women with polycystic ovary syndrome. Journal of Human Nutrition and Dietetics. 2018;31(2):266-75.

26. Grundy SM. Multifactorial causation of obesity: implications for prevention. Am J Clin Nutr. 1998 Mar;67(3 Suppl):563S-72S.

27. Tzotzas T, Vlahavas G, Papadopoulou SK, Kapantais E, Kaklamanou D, Hassapidou M. Marital status and educational level associated to obesity in Greek adults: data from the National Epidemiological Survey. BMC Public Health. 2010 Nov 26;10:732.

28. Wardle J, Waller J, Jarvis MJ. Sex differences in the association of socioeconomic status with obesity. Am J Public Health. 2002 Aug;92(8):1299-304.

29. Ferreira LG, Santos LF, Anastácio LR, Lima AS, Correia MITD. Resting Energy Expenditure, Body Composition, and Dietary Intake. Transplantation Journal. 2013;96(6):579-85.

30. Levitsky J, Singhvi A, Sadowsky HS, et al. Resting and Exercise Energy Metabolism After Liver Transplantation for Nonalcoholic Steatohepatitis. Transplant Direct. 2017 Aug;3(8):e188.

31. Perseghin G, Mazzaferro V, Benedini S, et al. Resting energy expenditure in diabetic and nondiabetic patients with liver cirrhosis: relation with insulin sensitivity and effect of liver transplantation and immunosuppressive therapy. Am J Clin Nutr. 2002 Sep;76(3):541-8.

32. Harris JA, Benedict FG. A biometric study of basal metabolism in man. Washington,: Carnegie Institution of Washington; 1919.

33. Peng S, Plank LD, McCall JL, Gillanders LK, McIlroy K, Gane EJ. Body composition, muscle function, and energy expenditure in patients with liver cirrhosis: a comprehensive study. Am J Clin Nutr. 2007 May;85(5):1257-66.

34. Flancbaum L, Choban PS, Sambucco S, Verducci J, Burge JC. Comparison of indirect calorimetry, the Fick method, and prediction equations in estimating the energy requirements of critically ill patients. Am J Clin Nutr. 1999 Mar;69(3):461-6.

35. Plevak DJ, DiCecco SR, Wiesner RH, et al. Nutritional support for liver transplantation: identifying caloric and protein requirements. Mayo Clin Proc. 1994 Mar;69(3):225-30.

36. Weijs PJ. Validity of predictive equations for resting energy expenditure in US and Dutch overweight and obese class I and II adults aged 18-65 y. Am J Clin Nutr. 2008 Oct;88(4):959-70.

37. Ebbeling CB, Swain JF, Feldman HA, et al. Effects of dietary composition on energy expenditure during weight-loss maintenance. JAMA. 2012 Jun 27;307(24):2627-34.

38. Westerterp KR. Physical activity and physical activity induced energy expenditure in humans: measurement, determinants, and effects. Front Physiol. 2013;4:90.

39. Ferraro R, Lillioja S, Fontvieille AM, Rising R, Bogardus C, Ravussin E. Lower sedentary metabolic rate in women compared with men. J Clin Invest. 1992 Sep;90(3):780-4.

40. Fukagawa NK, Bandini LG, Young JB. Effect of age on body composition and resting metabolic rate. Am J Physiol. 1990 Aug;259(2 Pt 1):E233-8.

41. Zhang K, Sun M, Werner P, et al. Sleeping metabolic rate in relation to body mass index and body composition. Int J Obes Relat Metab Disord. 2002 Mar;26(3):376-83.

42. Ravussin E, Burnand B, Schutz Y, Jequier E. Twenty-four-hour energy expenditure and resting metabolic rate in obese, moderately obese, and control subjects. Am J Clin Nutr. 1982 Mar;35(3):566-73.

43. Gallagher D, Visser M, Wang Z, Harris T, Pierson RN, Jr., Heymsfield SB. Metabolically active component of fat-free body mass: influences of age, adiposity, and gender. Metabolism. 1996 Aug;45(8):992-7.

44. Gabe SM, Bjarnason I, Tolou-Ghamari Z, et al. The effect of tacrolimus (FK506) on intestinal barrier function and cellular energy production in humans. Gastroenterology. 1998 Jul;115(1):67-74.

45. Plank LD, Metzger DJ, McCall JL, et al. Sequential changes in the metabolic response to orthotopic liver transplantation during the first year after surgery. Ann Surg. 2001 Aug;234(2):245-55.

46. Illsinger S, Goken C, Brockmann M, et al. Effect of tacrolimus on energy metabolism in human umbilical endothelial cells. Ann Transplant. 2011 Apr-Jun;16(2):68-75.

47. Palacin M, Coto E, Llobet L, Pacheu-Grau D, Montoya J, Ruiz-Pesini E. FK506 affects mitochondrial protein synthesis and oxygen consumption in human cells. Cell Biol Toxicol. 2013 Dec;29(6):407-14.

48. Utaka S, Avesani CM, Draibe SA, Kamimura MA, Andreoni S, Cuppari L. Inflammation is associated with increased energy expenditure in patients with chronic kidney disease. Am J Clin Nutr. 2005 Oct;82(4):801-5.

49. Michael Kjaer JJ, Susanne Keiding, Henrik Galbo, Preben Kirkegaard and Esther Hage No reinnervation of hepatic sympathetic nerves after liver transplantation. Journal of Hepatology. 1994;20:97-100.

50. Blaszkiewicz M, Townsend KL. Adipose Tissue and Energy Expenditure: Central and Peripheral Neural Activation Pathways. Current Obesity Reports. 2016;5(2):241-50.

51. Vijgen GH, Bouvy ND, Leenen L, et al. Vagus nerve stimulation increases energy expenditure: relation to brown adipose tissue activity. PLoS One. 2013;8(10):e77221.

52. Boon AP, Hubscher SG, Lee JA, Hines JE, Burt AD. Hepatic reinnervation following orthotopic liver transplantation in man. J Pathol. 1992 Jun;167(2):217-22.

53. Dhillon AP, Sankey EA, Wang JH, et al. Immunohistochemical studies on the innervation of human transplanted liver. J Pathol. 1992 Jun;167(2):211-6.

54. Livingstone MB, Black AE. Markers of the validity of reported energy intake. J Nutr. 2003 Mar;133 Suppl 3:895S-920S.

55. Lichtman SW, Pisarska K, Berman ER, et al. Discrepancy between self-reported and actual caloric intake and exercise in obese subjects. N Engl J Med. 1992 Dec 31;327(27):1893-8.

56. Yang YJ, Kim MK, Hwang SH, Ahn Y, Shim JE, Kim DH. Relative validities of 3-day food records and the food frequency questionnaire. Nutr Res Pract. 2010 Apr;4(2):142-8.

5. CAPÍTULO 2: Immunosuppression determines a lower response to a weight loss dietary intervention in liver recipients

Este artigo será enviado para publicação na revista *The Journal of Nutrition*, fator de impacto 4.145, Qualis A1 em Ciência de Alimentos.

Immunosuppression determines a lower response to a weight loss dietary intervention

Débora Fernandes Rodrigues[1], Gabriela Barbosa Pires Fagundes[1], Brenda Loise Monteiro[1], Nayara Mussi Monteze[1], Ana Maria dos Santos Rodrigues[1], Érica Leandro Marciano Vieira[2], Antônio Lúcio Teixeira[2], Mauro Martins Teixeira[3], Maria Isabel Toulson Davisson Correia[4], Simone de Vasconcelos Generoso[1], Adaliene Versiani Matos Ferreira[1]

[1] Immunometabolism, Department of Nutrition; Nursing School; Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil
[2] Department of Internal Medicine, Faculty of Medicine, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[3] Immunopharmacology, Department of Biochemistry and Immunology, Institute of Biological Sciences, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil.
[4] Alpha Institute of Gastroenterology; Hospital das Clínicas, Universidade Federal de Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Short running head: Immunosuppression influences weight loss

Key words: weight loss, immunosuppression, obesity, cytokines, liver transplantation.

Corresponding author: Adaliene Versiani Matos Ferreira. E-mail: adaliene@gmail.com. Phone: +5531 34098036. Department of Nutrition, Nursing School, Universidade Federal de Minas Gerais. Av. Prof. Alfredo Balena, 190 - Santa Efigênia, Belo Horizonte, Minas Gerais, Brazil. Zip Code: 30130-100.

Sources of support: Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Pró-Reitoria de Pesquisa da UFMG and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Clinical trial registry: ClinicalTrials.gov identification number: NCT03103984

INTRODUCTION

Obesity is defined as an excessive fat accumulation that can induces a chronic low-grade inflammation [1]. During the adipose tissue expansion, immune cells are recruited and start to produce a wide range of inflammatory mediators that, in turn, impairs metabolic health [2, 3]. On the other hand, recent evidences have shown that the adipose tissue inflammation is required to proper adipose tissue remodeling upon a dietary challenge [4], indicating that it is not all bad. Indeed, our group and others have shown that the immune and the metabolic systems have a complementary role in the control of the adipose tissue expansion and remodeling [5-7]. Platelet-activating factor, a molecule produced by several cell types, including leukocytes, was shown to be an important regulator of fat accumulation in adipocytes, mainly by modulating the inflammatory milieu of the adipose tissue [6]. Likewise, other studies showed that mice lacking interleukin (IL) 6 and tumor necrosis factor (TNF) signaling develop obesity even when eating standard diets [7, 8]. In line with this, Asterholm et al. [4] developed three mouse models with impaired adipocyte pro-inflammatory response and showed that, upon a high fat diet intake, the expansion of visceral adipose tissue was compromised, leading to high ectopic lipid accumulation, glucose intolerance and systemic inflammation. The authors concluded that an adaptive response is required to limit the adipose tissue over expansion and to enable safe storage of excess nutrients.

Accordingly, human studies have also shown that an impaired inflammatory response leads to weight gain. Significant increase in body weight has been extensively reported in patients treated with immunosuppressive agents [9-14], but the mechanism of such weight gain is still unknown. Interestingly, immunosuppressed patients who have undergone different solid organ transplantation, such as kidney, heart, lung, and liver frequently gain excessive body weight [15, 16]. Specifically, around 80% of liver recipients have some degree of abdominal obesity 4 years after transplantation [17]. The body weight gain after liver transplantation (LT) is likely to be multifactorial, including changes in energy intake [18], the use of immunosuppressive agents [19], and low resting energy expenditure (REE) [20, 21], but there is still no consensus in the literature. The increase in body mass index (BMI) after LT is sometimes attributable to the use of corticosteroids as part of the immunosuppressive therapy [22], since these drugs have been reported to increase appetite and alter fat metabolism [23]. However, liver recipients continue to gain weight after steroid withdrawal [17].

There are several studies linking the inflammatory and the metabolic responses, but there is still much debate regarding the physiological role of the immune system in obesity. Interestingly, the belief that inflammatory signals exert a fundamentally negative impact on metabolism may not be totally true since inflammation may also be required for proper adipose tissue remodeling. To better understand the crosstalk between these two systems, we studied immunosuppressed patients to answer our major question: does the immune system plays a pivotal role during the weight loss? As the inflammatory response is an important regulator of the adipose tissue remodeling, we hypothesized that liver recipients would not successfully respond to a weight loss nutritional planning. Thus, the aim of the present study was two-fold: (i) to characterize the immune cell profile of overweight liver recipients, and (ii) to evaluate whether those patients are less responsive to a weight loss dietary intervention.

METHODS

Participants

This study was approved by the ethics committee of the Universidade Federal de Minas Gerais, Brazil (CAAE: 30409114.8.0000.5149) and was registered in the Clinical Trial Register under the ClinicalTrials.gov Identifier NCT03103984.

Between August 2014 and May 2016, liver recipients were recruited from the outpatient practices of the Hospital das Clínicas/ Universidade Federal de Minas Gerais. Patients included have 18-65 years of age, a BMI $\geq$ 25 kg/m^2 and 1-3 years of LT, when many of them are overweight/ obese and adapted to the first-year post-transplantation [24, 25]. The exclusion criteria were: pregnancy, breast-feeding, re-graft, use of hormonal therapy or weight loss medication, and external nutritional counseling already in progress.

Liver recipients were paired with non-transplanted subjects in respect to sex, age and BMI to provide accurate comparisons. Subjects from this group followed the same inclusion/ exclusion criteria applied to the LT group, except they were not in use of immunosuppressive or anti-inflammatory drugs. Bariatric patients were left out. Those who have a BMI $\geq$ 40 kg/m^2 were excluded to avoid discrepancy from LT group and allow comparisons.

This study was divided into two phases: first, we characterized the peripheral immune profile of the volunteers (first described below), and second, subjects were instructed to follow a nutritional planning to provide weight loss (described subsequently). The study design is detailed in **Figure 1**.

Peripheral immune profile of the subjects

Peripheral blood isolation and cell surface staining: blood was collected in the morning at the same day of clinical interview. The blood was drawn and immediately taken to the laboratory and processed. Whole blood cells were obtained from K3-EDTA venous vacuum tubes. Peripheral blood mononuclear cells (PBMC) were obtained after Ficoll gradient centrifugation using Ficoll-Hystopaque Plus (GE Healthcare, MA, USA). The PBMC were stained with a combination of fluorescein isothiocyanate (FITC), phycoerythrin (PE), cy5.5- chrome (Cy)-labeled or PerCP 5.5, allophycocyanin (APC), cy7- allophycocyanin (APC) and cy7-phycoerythrin (PE-Cy7) antibodies directed against the surface molecules anti-CD3, anti-CD4, anti-CD8, anti-CD25, anti-CD69, anti-CD56, anti-CD19, anti-CD14 and anti-CD16 (eBioscience, San Diego, CA, USA; BDPharMingen, San Diego, CA, USA and Invtrogen/ Molecular Probes, Camarillo, CA, USA) for 20 minutes at 4ºC. Data were acquired using a FACSCanto II (Becton & Dickinson, San Jose, CA, USA).

Intracellular cytokines and FoxP3 staining: PBMC were analyzed for their surface profile and intracellular cytokine expression pattern. Briefly, cells were fixed with phosphate buffer saline (PBS) and formaldehyde (2%) (Sigma-Aldrich, St. Louis, MO, USA) for 20 minutes. Fixed cells were permeabilized using saponin 0,5% (Sigma-Aldrich) and stained using monoclonal antibodies for FoxP3, TNF, IL-6, IFN-γ, IL-17 and IL-10 (Invitrogen/Molecular Probes and BDPharmingen) conjugated with phycoerythrin (PE) or allophycocyanin (APC). PE and APC-labeled immunoglobulin control antibodies and a control of unstaining PBMC were also included in all experiments. Preparations were acquired in FACSCanto II (Becton & Dickinson, San Jose, CA, USA). A minimum of 100,000 gated events in lymphocyte population was acquired for analysis due to the low frequency of positive events being analyzed.

Flow cytometry data analysis: Natural killer (NK), T and B lymphocytes were analyzed for their intracellular cytokine and costimulatory surface marker expression pattern and frequency using the FlowJo program (Tree Star, Ashland, OR, USA). Limits for the quadrant markers were always set based on negative populations and isotype controls. At least three different fluorochromes were combined for each analysis. After gating specific cell subsets, histograms were generated for evaluating the frequency of cells expressing the given surface markers or cytokines. These cells were then analyzed for the expression (frequency and mean fluorescent intensity, MFI) of a given marker using histograms with control markers set based on negative isotype controls.

PBMC culture: PBMC were used for in vitro assays under different stimuli: lectin from *Phaseolus vulgaris* (PHA) (Sigma-Aldrich) at 1%, lipopolysaccharides (LPS) from *Escherichia coli* (Sigma-Aldrich) at 10µg/ mL, and anti-CD3/ anti-CD28 (10µg/ mL and 1µg/mL, respectively) (BD Pharmingen). PBMC (2x105 cells/ well) were maintained in RPMI media, supplemented with 10% male human serum and 1% antibiotic-antimycotic solution (Sigma-Aldrich), in 5% of CO_2 incubator at 37ºC for 24 hours. After the culture, the plate was centrifuged (1200 rpm, 10 minutes at 4ºC) and the supernatant was collected. The supernatant was used for Cytometric Bead Array (CBA). Human Th1/ Th2/ Th17 kit (Becton & Dickinson) was used. We evaluated the cytokines Interferon (IFN) γ, TNF, IL-2, IL-4, IL-6, IL-10 and IL-17 following manufacturer's instructions. Briefly, capture beads for each protein was centrifuged together (200 g for 5 minutes), re-suspended in plasma enhancement buffer and incubated for 30 minutes at room temperature. After, 50 µl of this solution containing the capture beads was added to the assay tubes. Lyophilized standards were reconstituted (15 minutes at room temperature) for serial dilution. Individual samples from subjects or standards were added (50 µl) to the assay tubes. Human PE detection reagent (50 µl) was added to all assay tubes, which were incubated for 3 hours at room temperature. Then, wash buffer (1 ml) was added to each assay tube and centrifuged at 200 g for 5 minutes. The supernatant was discarded, and 300 µl of wash buffer was added to each assay tube again to re-suspend the bead pellet. Finally, all samples and standards were acquired on the flow cytometer (FACS CANTO II). The results were analyzed using FCAP Array software (Becton & Dickinson, San Jose, CA, USA).

Cytokines serum concentrations: Blood samples were obtained from subjects in the fasted state and centrifuged to obtain the serum, which was aliquoted and stored at −80 °C until analysis. IFN-γ, TNF, IL-2, IL-4, IL-6, IL-10 and IL-17 were assessed in the sera using a Th1/ Th2/ Th17 cytokine kit (BD Biosciences, CA, USA) by CBA. The adipokines leptin, adiponectin and resistin were assessed using Enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Abingdon, UK). Both methods were performed according to the manufacturers' instructions.

Nutritional prescription and monitoring

All subjects received individualized counseling for dietetic modification provided by a nutritionist. They were instructed to follow a diet (50% carbohydrates, 30% lipids, 20% proteins) with a caloric restriction to provide 5-10% of weight loss after 6 months. The expected weight loss was calculated considering the Wishnofsky constant (7700 kcal kg^{-1}) [26]. The first counseling session lasted approximately 2 h and included a verbal review of subjects' data, such as their baseline assessments and nutritional diagnosis, and the short and long-term goal setting. To maximize adherence, all subjects received monthly follow-up counseling in person at the clinic. The dietary prescription was adjusted according to changes in anthropometric parameters, REE and physical activity level. Their daily activities and routine were converted into Metabolic Equivalents (MET), as described elsewhere [21], to adjust the total caloric value of theirs diets.

Each follow-up counseling session included a review of the goal setting, suggestions for changes, encouragement to continue participating in the study and assessment of the adherence to the proposed dietary habits. The last was done by applying a self-completed questionnaire as proposed elsewhere [27].

Anthropometric measurements: anthropometric measurements were performed in the baseline and in the subsequent meetings (1^{st}, 2^{nd}, 3^{rd} and 6^{th} months of follow-up). Body weight and height were obtained using a floor model physician's scale/ stadiometer (Filizola®). BMI was calculated and classified according to World Health Organization (WHO) criteria [28]. The waist circumference was made at the level of the umbilicus

[29]. Multifrequency bioelectrical impedance analysis (Quantum X - RJL Systems, Inc., Clinton Township, Michigan) was used to determine body composition and were performed during the morning, after an overnight fasting, with the volunteers in the supine position.

Resting Energy Expenditure (REE): the REE was assessed by indirect calorimetry, using an open-circuit calorimeter (MetaCheck™ metabolic rate analysis system, model 7100, Korr Medical Technologies). The participants attended the clinic in the morning, in the fasted state, and remained seated for 30 minutes before the evaluation. The measurements were made in a temperature-controlled room (22-24 ^{o}C) during 10 minutes.

Dietary intake: subjects were requested to fill in a 3-day food record in the baseline and in the subsequent meetings. We asked volunteers to complete it in the week before the meeting, considering 3 non-consecutive days and 1 weekend day. Food intake data was entered using the software Brasil Nutri® and converted into calories, carbohydrates, proteins and lipids using tables of food composition [30, 31]. These data provided information about their dietary habits before and after the nutritional intervention.

Metabolites serum concentrations: blood samples were obtained from subjects in the fasted state and centrifuged to obtain the serum, which was aliquoted and stored at −80°C until analysis. Total and HDL cholesterol, triglycerides and glucose concentrations were assayed in the sera using enzymatic kits (Bioclin, Belo Horizonte, MG, Brazil). The LDL and VLDL serum concentrations were obtained through equations available in the manufacturers' protocol.

Statistics

Data were analyzed with the Statistical Package for the Social Sciences (SPSS) software, version 19.0 (IBM Corporation, Armonk, NY, USA). For descriptive purposes, categorical variables were compared using chi-square analyses. All data were analyzed for the normality of their distributions using the Kolmogorov-Smirnov test. Continuous variables taken at different time intervals were compared by Generalized

Estimating Equation (GEE) Model to evaluate the effect of group allocation, adjusting for time effect (group*time). The variables were treated with a connection linear or gamma function according to the type of distribution. The working correlation matrix used was unstructured and robust estimator covariance matrix. For significant effects we used post-hoc Bonferroni. Correlation between weight loss percentage and tacrolimus serum concentration were calculated with Spearman's coefficient. Differences were considered statistically significant at the $P < 0.05$ level (symbols: * was used for intra-group differences and $^{\#}$ for differences in interaction C x LT).

The sample size was estimated in 17 participants to detect a 5% reduction of weight. We considered a traditional effect size of 0.5, a statistical power of 80%, an alpha level of 0.05, and a correction for the total sample of eligible patients (n=34). To characterize the peripheral immune profile of liver recipients, a subsample of 14 patients (7 from each group) were conveniently included.

RESULTS

Subjects characterization

Out of 100 patients who had completed 1-3 years of LT in the first meeting of the follow-up period, eighty were excluded due to the following reasons: re-graft (n=10), age less than 18 (n=4) or more than 65 years old (n=13), BMI< 25 kg/m^2 (n=30), insulin use (n=7), lack of interest (n=14) and external weight loss program in progress (n=2). Thus, a total of 20 patients met the inclusion criteria and accepted to participate in the study. During the follow-up period, nine liver recipients abandoned the intervention and 11 completed the study (**Figure 2**). A total of 83 subjects were interest in enrolling the control group, but only 50 fitted the inclusion criteria. In the course of the study, twenty-nine declined to participate and 2 got pregnant, thus nineteen subjects completed the study (**Figure 2**).

Baseline characteristics of the subjects are listed in **Table 1**. Most of the volunteers from both groups were male (C: n=11; 57.9%/ LT: n=9; 81.8%; $P > 0.05$) and have a similar mean age (C: 42 ± 1.9/ LT: 49 ± 3.6 years old; $P > 0.05$). There was no difference between the socioeconomic data of both groups, such as marital status and family income. LT patients and controls practiced the same level of physical activity,

and the majority of them are sedentary (C: n=11; 57.9%/ LT: n=7; 63.6%; $P > 0.05$). Because both groups were carefully paired, there was no difference in the BMI of the volunteers (C: 31.0 ± 0.75/ LT: 28.9 ± 0.98 kg/m^2; $P > 0.05$). The REE of the liver recipients were different from controls (C: 1,877.0 ± 98.8/ LT: 1,527.2 ± 122.3 kcal; $P < 0.05$) (**Table 1)**.

The average time since LT was 23 ± 3 months (**Table 1**). The patients have undergone LT owing to alcohol abuse (45.5%), hepatitis C virus (27.2%), cryptogenic cirrhosis (18.2%) and autoimmune hepatitis (9.1%). All liver recipients were in use of tacrolimus and only 1 were simultaneously using corticosteroids.

Immunosuppression characterization

To better understand whether the immunosuppressed state influences weight loss in overweight liver recipients, we first characterized peripheral immune cells and their activation status in liver recipients (n=7) and control subjects (n=7) in the baseline. The groups showed the same immune cell profile regarding the percentage of total lymphocytes (Total CD3+), the subtypes T helper (CD3+CD4+), Treg (CD3+CD4+CD25high), cytotoxic T cells (CD3+CD8high), natural killer (NK) cells (CD8lowCD56+); and monocytes (Total CD14+) (**Table 2**). However, in the LT group, the frequency of B lymphocytes (CD3-CD19+) were higher, and the NK cells expressing the CD16+ receptor were decreased. Regarding the other markers evaluated, including intracellular cytokines and costimulatory surface molecules expression, there was no difference among the groups.

Liver recipients showed altered inflammatory milieu compared to control subjects in the basal state. The levels of IFNγ, TNF, IL-4, IL-2 and IL-10 in the sera were decreased in the LT group, whereas there was no difference in the IL-17 and IL-6 levels (**Figure 3A**). There was no difference among the adipokines adiponectin, resistin and leptin in the sera (**Figure 3B**) when comparing both groups.

We next evaluated the inflammatory response in cultured PBMC after different stimuli: LPS, anti-CD3/anti-CD28, and PHA (**Figure 4**). PBMC from control subjects were responsive to LPS, increasing the production of TNF, IL-6 and IL-10 (**Figure 4A-C**); to anti-CD3/anti-CD28, increasing IL-6, IL-4, IL-17, IFN-γ and IL-2 levels (**Figure 4 B, D-G**); finally, they respond to PHA, showing high levels of TNF, IL-6, IL-10 and

IL-17 (**Figure 4 A-C, E**). In turn, liver recipients' PBMC were responsive to LPS, increasing the production of TNF and IL-6 (**Figure 4 A, B**); to anti-CD3/anti-CD28, through high levels of IL-4 and IL-2 (**Figure 4 D, G**); and to PHA, increasing TNF, IL-6 and IFN-γ concentrations (**Figure 4 A, B, F**). When comparing both groups, liver recipients showed lower levels of IL-4 and IL-2 in media condition, as under LPS stimulus (**Figure 4 D, G**). After incubation with anti-CD3/anti-CD28, PBMC from LT subjects were less responsive than controls regarding the production of IL-10 and IL-17 (**Figure 4 C, E**). The production of IL-17 were also low after exposure to PHA in liver recipients' PBMC (**Figure 4 E**).

Nutritional monitoring and weight loss

According to the self-reported questionnaire, both groups showed the same level of dietary adherence after 3 and 6 months following the nutritional prescription (**Table 3**). Considering the first three sentences of the questionnaire, most of the subjects from control and LT groups adhered to the majority of the orientations until the third and sixth months of follow-up.

After 3 and 6 months, subjects from control group lost, in average, 1.77% and 3.73% of weight, respectively. The weight loss in the LT group was lower than controls (3 months: 0.42%; 6 months: 0.54%) (**Table 4**). In accordance, control subjects improved their body composition after the dietary intervention, including reduction in body weight, body fat mass and waist circumference. No changes were found in fat-free mass (**Table 4**). Liver recipients did not change their body composition.

The energy intake and the dietary macronutrient content of the groups were unchanged along the follow-up period, considering both inter and intra-group comparations (**Table 5**).

The weight loss of both groups was not enough to drive significant modifications in serum metabolites, as total cholesterol, lipoprotein fractions, triglycerides and glucose (**Table 6**). Liver recipients showed low levels of total and LDL cholesterol when compared to controls at the baseline. This difference was maintained for total cholesterol until 3 months of follow up.

Given that liver recipients did not lose body weight as the control group did, we investigated whether the immunosuppressant therapy influenced this response. The

Spearman correlation between body weight loss percentage and tacrolimus serum concentration showed an inverse association, suggesting a key role of the immunosuppressive state driven by tacrolimus in the weight loss process (**Figure 5**).

The weight loss dietary intervention lead to a reduction in the serum cytokines from control subjects, such as IL-4, IL-2, IFN-γ, IL-17, IL-10, IL-6 and resistin, as evidenced by their negative variation in the follow-up period (**Figure 6**), which was not observed in the liver recipients. There was no difference in the serum TNF, leptin and adiponectin variation when comparing both groups.

DISCUSSION

Obesity is often characterized by an over-expanded and inflamed adipose tissue, resulting in a state of chronic low-grade inflammation. However, how this dysregulated immune response affects whole body homeostasis and the development of obesity has been the topic of intense investigation. In the present study, we tested the hypothesis that the immune system plays a pivotal role in the weight loss process and we showed that immunosuppressed liver recipients did not successfully respond to a weight loss nutritional intervention.

It has been postulated that the inflammatory response exerts a physiological role to control the expansion or the reduction of the adipose tissue [32]. In fact, inflammatory cells, such as macrophages, CD4+, CD8+, Treg and B cells resides in the adipose tissue [33-36]. The accumulation of these immune cells into the fat-pad is pathologically accelerated in obese individuals and it is characterized by a dramatically stromal cell change in number and cell type during the course of obesity [37]. This process might be implicated in the relationship between the inflammatory and metabolic parameters [38]. To better understand the crosstalk between these both systems, we first characterized the peripheral immune cell profile of the subjects. The frequency of total monocytes, total lymphocytes, and the subtypes T helper, Treg, and cytotoxic T cells did not differ among the groups, except the frequency of B lymphocytes and $CD8^{low}$CD56+CD16+ NK cells. It is known that tacrolimus acts more selectively at the level of T cells [39], influencing B cells proliferation depending on the dosage and type of stimulation [39, 40]. Like T cells and macrophages, B cells have also been shown to play important roles in the obesity-related inflammation, since they infiltrate into the

expanding adipose tissue, release inflammatory cytokines and chemokines, and regulate the T cell function [35, 41]. Studies elucidating B cell function in obesity are scarce, but Frasca et al. [42] have recently shown that peripheral B lymphocytes of obese subjects is characterized by low percentages of anti-inflammatory B cell subsets, high percentages of proinflammatory memory B cells, and an impaired B cell function.

The frequency of $CD8^{low}$CD56+CD16+ NK cells were decreased in the liver recipients. This subset of lymphocytes plays an important role in recognizing and killing a variety of virally infected or tumor cells without prior antigen sensitization [43]. The CD16 receptor was found to activate the cell-mediated cytotoxicity [44]. Lynch el at. [45] showed that invariant NK cells are enriched in human adipose tissue, but are decreased in human obesity. Later, these authors showed that mice lacking invariant NK cells gained more weight than wild type mice on a high fat diet, and had larger adipocytes, fatty livers and were insulin resistant [46]. Though primarily considered as part of the innate immune response, NK cells produce cytokines such as IFN-γ and TNF [47]. Herein, we showed that these cytokines are reduced in LT patients. IL-4 and IL-10, potent inhibitors of pro-inflammatory cytokines [48], and IL-2, a tacrolimus-target cytokine [49], are also reduced. In lean individuals, it is thought that a wide-ranging cytokines' producing cells, such as innate and adaptive immune cells, dominate in the adipose tissue to ensure homeostasis [34, 50, 51]. Thus, we believe that the impairment in the inflammatory milieu due to the immunosuppressed state may account for a disturbed control in the gain and loss of body weight in liver recipients.

Besides the low systemic levels of inflammatory cytokines, liver recipients' PBMC were less responsive to the production of IL-4, IL-2, IL-10 and IL-17 when compared to controls after the incubation with different stimuli. Similar findings were demonstrated by Howell et al. [52], who found that there was an impairment in proinflammatory cytokine production by PBMC from liver recipients after different Toll-like receptor stimulation. It is not surprising, since tacrolimus is a calcineurin inhibitor and it thereupon prevents the dephosphorylation of the nuclear factor of activated T cells, its nuclear translocation and interaction with different genes, including those encoding the IL-2 and its receptor [49]. Consequently, it reduces the immunocompetence to prevent organ rejection after transplantation.

As such, tacrolimus is widely used in the immunosuppressive therapy after LT [53] owing to its beneficial effects compared to other immunosuppressants, like cyclosporine, including lower acute rejection, better glomerular filtration rate and better

lipid profile [54, 55]. Nevertheless, it has been reported that liver recipients gain excessive body weight after LT [56], which has been associated with the immunosuppressive therapy [14]. However, it is still controversial and other factors have been implicated in such weight gain. In the present work, we found that liver recipients are hypometabolic, in accordance with our previous data [21]. Although the hypometabolism could partially explain the high prevalence of overweight and obesity after liver transplantation, it could not explain why liver recipients did not lose weight after the dietary intervention. Herein, the LT patients followed a nutritional planning for 6 months to lose weight. During this period, the REE of the volunteers was measured every meeting and the diet was properly adjusted to it to remove the bias of the hypometabolic state. Nevertheless, liver recipients did not lose weight as the control group did, even with the same adherence to the nutritional planning. Thus, we investigated whether the immunosuppressed state might be interfering in the weight loss process in liver recipients.

Considering the low systemic levels of inflammatory cytokines in the LT group, and the crosstalk between inflammation and the adipose tissue, we correlated the serum tacrolimus concentration with the mean weight loss percentage and we found an inverse correlation. We and others [6, 57] have previously shown that an effective immune response is of crucial importance to control adiposity and to drive weight loss. Lacerda et al. [58] showed that fasting leads to increased leukocyte influx and cytokines concentrations in murine adipose tissue, such as TNF, IL-6, IL-10 and TGF-β, highlighting that inflammatory mediators may have a physiological purpose to mediate adipose tissue remodeling. Kosteli et al. [57] also showed that fasting and lipolysis lead to a rapidly increase in macrophage accumulation and lipid uptake in the adipose tissue of mice, suggesting that these immune cells may thus protect local adipocyte function. García et al., [59], Menezes-Garcia et al. [6] and Martins et al. [7] supported this idea by demonstrating the development of obesity in mice lacking cytokine receptors. These authors showed that the absence of some cytokines signaling leads to significant gain of adiposity, suggesting that inflammation has a pivotal role in the development of obesity. Clinical studies have also clearly showed the effect of different immunosuppressive therapies on body weight gain in patients with different diseases, such as psoriasis [11], rheumatoid arthritis [10], acquired immunodeficiency syndrome [12], and Crohn's disease [13]. In the last one, the authors found an 18% increase in total abdominal fat

volume in the patients treated with anti-TNF. In fact, TNF is a powerful regulator of adipose tissue metabolism. It increases lipolysis in adipocytes by regulating lipid droplet-associated proteins and lipases in adipocytes [60], thus altering body fat mass. Likewise, Emilie et al. [12] studied the effect of an anti-IL-6 monoclonal antibody in 11 subjects seropositive for human immunodeficiency virus-l. After 21 days of the antibody administration, they observed an average weight gain of 1.4 ± 0.5 kg.

In controls, the systemic reduction in the inflammatory milieu after nutritional intervention can be associated with the decrease in the adipose tissue mass and, consequently, its production of inflammatory mediators. Control subjects improved their body composition after the dietary intervention, since they showed a decrease in the body weight and fat, waist circumference, and maintained their fat-free mass. Clément et al. [61] showed that obese subjects improved their inflammatory profile after weight loss through a decrease of proinflammatory factors and an increase of anti-inflammatory molecules. They concluded that the positive effect of weight loss on obesity-related disorders may be associated with the change of the inflammatory profile localized in the adipose tissue. Other studies also showed that weight loss improved serum cytokines in obese subjects [62, 63]. The very low weight loss percentage of liver recipients did not alter the systemic cytokines levels.

We no longer aimed to elucidate the role of specific cytokines in the weight loss process, but, instead, our study brings up the idea that the inflammatory milieu might play a pivotal role in the weight loss process. We hypothesized that the inflammatory pathways physiologically triggered to control adipose tissue remodeling are switched off under a disturbed inflammatory response, as occurs in liver recipients.

The present work has some limitations. First, we studied only liver recipients to understand the role of the immune system in the weight loss process, which limit our data to this population. Second, adherence rates to weight loss programs is critical to define the successful of the intervention, but it is difficult to measure [64, 65]. Herein, we applied a self-completed questionnaire [27], and we provided monthly follow-up counseling in person at the clinic, encouraging participants to achieve weight loss. Self-reported questionnaires have been related as the most viable option in most studies, but the dialogue have been considered the best approach to assess patient adherence [65]. Third, due to methodological and ethical limitations surrounding researches involving human subjects, we were unable to locally evaluate the adipose tissue inflammatory response, which could improve the understanding about the fat-pad remodeling. Finally,

the underreporting of dietary intake is commonly seen in overweight subjects [66]. Nevertheless, the 3-day food record applied here to estimate energy and macronutrient intake has been widely used in different works [67, 68] and shows high validity [69].

Despite the limitations, this work provides a set of results that represents one step forward in the knowledge about the interface between immune and metabolic system, particularly by studying liver recipients. Although the number of participants in this study was not large, a great merit herein was to keep a statistically feasible number of patients until the end of the study. Many liver recipients live in different cities away from the clinic, which is an important dropout factor. Likewise, weight loss studies are challenging and demands a complex behavior change [64], what makes many subjects to give up.

In summary, LT patients showed minor differences regarding the frequency of immune cells when compared to controls, highlighting only low and high levels of $CD8^{low}CD56^{+}CD16^{+}$ NK and B cells, respectively. On the other hand, liver recipients showed lower systemic levels of cytokines and a lower responsiveness to different inflammatory stimuli. This impaired inflammatory milieu might be implicated in the lack of response to the weight loss dietary intervention. Our results add novel insights about the link between the immune system and the weight control.

ACKOWNLEDMENTS

We thank the volunteers who participated in this study, as well as the personnel of the Hospital das Clínicas laboratory for the blood samples collection. We are very grateful to our colleague Jenneffer Rayane Braga Tibães for assisting in the analysis with the software Brasil Nutri®.

Funding: this study was financed by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001; Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG); Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq); and Pró-Reitoria de Pesquisa da UFMG.

REFERENCES

1. Tateya, S., F. Kim, and Y. Tamori, *Recent advances in obesity-induced inflammation and insulin resistance.* Front Endocrinol (Lausanne), 2013. **4**: p. 93.

2. Piche, M.E. and P. Poirier, *Obesity, ectopic fat and cardiac metabolism.* Expert Rev Endocrinol Metab, 2018. **13**(4): p. 213-221.

3. Gilani, A., et al., *High Fat Diet-Induced Obesity and Insulin Resistance in Cyp4a14(-/-) Mice Is Mediated by 20-Hete.* Am J Physiol Regul Integr Comp Physiol, 2018.

4. Wernstedt Asterholm, I., et al., *Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling.* Cell Metab, 2014. **20**(1): p. 103-18.

5. Rodrigues, D.F., et al., *Acute intake of a high-fructose diet alters the balance of adipokine concentrations and induces neutrophil influx in the liver.* J Nutr Biochem, 2014. **25**(4): p. 388-94.

6. Menezes-Garcia, Z., et al., *Lack of platelet-activating factor receptor protects mice against diet-induced adipose inflammation and insulin-resistance despite fat pad expansion.* Obesity (Silver Spring), 2014. **22**(3): p. 663-72.

7. Martins, L.B., et al., *Paradoxical role of Tumor Necrosis Factor on metabolic dysfunction and adipose tissue expansion in mice.* Nutrition, 2017.

8. Wallenius, V., et al., *Interleukin-6-deficient mice develop mature-onset obesity.* Nat Med, 2002. **8**(1): p. 75-9.

9. Saraceno, R., et al., *Effect of anti-tumor necrosis factor-alpha therapies on body mass index in patients with psoriasis.* Pharmacol Res, 2008. **57**(4): p. 290-5.

10. Brown, R.A., et al., *Long-term effects of anti-tumour necrosis factor therapy on weight in patients with rheumatoid arthritis.* Clin Rheumatol, 2012. **31**(3): p. 455-61.

11. Tan, E., C. Baker, and P. Foley, *Weight gain and tumour necrosis factor-alpha inhibitors in patients with psoriasis.* Australas J Dermatol, 2013. **54**(4): p. 259-63.

12. Emilie, D., et al., *Administration of an anti-interleukin-6 monoclonal antibody to patients with acquired immunodeficiency syndrome and lymphoma: effect on lymphoma growth and on B clinical symptoms.* Blood, 1994. **84**(8): p. 2472-9.

13. Parmentier-Decrucq, E., et al., *Effects of infliximab therapy on abdominal fat and metabolic profile in patients with Crohn's disease.* Inflamm Bowel Dis, 2009. **15**(10): p. 1476-84.

14. Charlton, M., et al., *Everolimus Is Associated With Less Weight Gain Than Tacrolimus 2 Years After Liver Transplantation: Results of a Randomized Multicenter Study.* Transplantation, 2017. **101**(12): p. 2873-2882.

15. Bianchi, G., et al., *Metabolic syndrome in liver transplantation: relation to etiology and immunosuppression.* Liver Transpl, 2008. **14**(11): p. 1648-54.

16. Kugler, C., et al., *Postoperative weight gain during the first year after kidney, liver, heart, and lung transplant: a prospective study.* Prog Transplant, 2015. **25**(1): p. 49-55.

17. Anastacio, L.R., et al., *Body composition and overweight of liver transplant recipients.* Transplantation, 2011. **92**(8): p. 947-51.

18. Roske, A.E. and M. Plauth, *Liver transplantation, body composition, and substrate utilization: does organ transplantation normalize the metabolic situation of the patient?* Nutrition, 1999. **15**(6): p. 504-5.

19. Neal, D.A., et al., *Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids, and weight.* Liver Transpl, 2001. **7**(6): p. 533-9.

20. Richardson, R.A., O.J. Garden, and H.I. Davidson, *Reduction in energy expenditure after liver transplantation.* Nutrition, 2001. **17**(7-8): p. 585-9.

21. Rodrigues, D.F.M., N.M.; Fagundes, G.B.P.; Monteiro, B.L.M.; Lima, A.S.; Correia, M.I.T.D.; Generoso, S.V.; Ferreira, A.V.M., *Hypometabolism as a potential risk factor for overweight and obesity in liver recipients* Ahead of print: Nutrition.

22. Rogers, C.C., et al., *Body weight alterations under early corticosteroid withdrawal and chronic corticosteroid therapy with modern immunosuppression.* Transplantation, 2005. **80**(1): p. 26-33.

23. Dallman, M.F., et al., *Minireview: glucocorticoids--food intake, abdominal obesity, and wealthy nations in 2004.* Endocrinology, 2004. **145**(6): p. 2633-8.

24. Rezende Anastacio, L., et al., *Overweight, obesity and weight gain up to three years after liver transplantation.* Nutr Hosp, 2012. **27**(4): p. 1351-6.

25. Richards, J., et al., *Weight gain and obesity after liver transplantation.* Transpl Int, 2005. **18**(4): p. 461-6.

26. Wishnofsky, M. *Caloric Equivalents of Gained or Lost Weight.* The American Journal Of Clinical Nutrition, *1958, 6(5).*

27. Toledo, M.T.T., Abreu, M.N. and Lopes, A.C.S. *Adesão a modos saudáveis de vida mediante aconselhamento por profissionais de saúde.* Revista de Saúde Pública, 2013. **47**(3): p. 540-548.

28. WHO, *Obesity: preventing and managing the global epidemic. Report of a WHO consultation.* World Health Organ Tech Rep Ser, 2000. **894**: p. i-xii, 1-253.

29. WHO, *Waist Circumference and Waist-Hip Ratio, Report of a WHO Expert Consultation*. 2011.

30. USDA, U.S. Department of Agriculture, *USDA Food Composition Databases.* 2018.

31. NEPA, *Tabela Brasileira de Composição de Alimentos – TACO 4ª edição revisada e ampliada.* Campinas, SP, 2011: p. 161.

32. Asterholm, W.I., et al., *Adipocyte Inflammation Is Essential for Healthy Adipose Tissue Expansion and Remodeling.* Cell Metabolism, 2014. **20**(1): p. 103-118.

33. Nishimura, S., et al., *CD8+ effector T cells contribute to macrophage recruitment and adipose tissue inflammation in obesity.* Nat Med, 2009. **15**(8): p. 914-20.

34. Feuerer, M., et al., *Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters.* Nat Med, 2009. **15**(8): p. 930-9.

35. Winer, D.A., et al., *B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies.* Nat Med, 2011. **17**(5): p. 610-7.

36. Winer, S., et al., *Normalization of obesity-associated insulin resistance through immunotherapy.* Nat Med, 2009. **15**(8): p. 921-9.

37. Tanaka, M., et al., *Molecular mechanism of obesity-induced 'metabolic' tissue remodeling.* J Diabetes Investig, 2017.

38. Feuerer, M., et al., *Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters.* Nature Medicine, 2009. **15**(8): p. 930-939.

39. Wasik, M., et al., *Effect of FK-506 and cyclosporine on human T and B lymphoproliferative responses.* Immunopharmacology, 1990. **20**(1): p. 57-61.

40. Morikawa, K., F. Oseko, and S. Morikawa, *The distinct effects of FK506 on the activation, proliferation, and differentiation of human B lymphocytes.* Transplantation, 1992. **54**(6): p. 1025-30.

41. DeFuria, J., et al., *B cells promote inflammation in obesity and type 2 diabetes through regulation of T-cell function and an inflammatory cytokine profile.* Proc Natl Acad Sci U S A, 2013. **110**(13): p. 5133-8.

42. Frasca, D., et al., *Obesity decreases B cell responses in young and elderly individuals.* Obesity (Silver Spring), 2016. **24**(3): p. 615-25.

43. Rosenberg, E.B., et al., *Lymphocyte cytotoxicity reactions to leukemia-associated antigens in identical twins.* Int J Cancer, 1972. **9**(3): p. 648-58.

44. Montaldo, E., et al., *Human NK cell receptors/markers: a tool to analyze NK cell development, subsets and function.* Cytometry A, 2013. **83**(8): p. 702-13.

45. Lynch, L., et al., *Invariant NKT cells and CD1d(+) cells amass in human omentum and are depleted in patients with cancer and obesity.* Eur J Immunol, 2009. **39**(7): p. 1893-901.

46. Lynch, L., et al., *Adipose tissue invariant NKT cells protect against diet-induced obesity and metabolic disorder through regulatory cytokine production.* Immunity, 2012. **37**(3): p. 574-87.

47. Andoniou, C.E., J.D. Coudert, and M.A. Degli-Esposti, *Killers and beyond: NK-cell-mediated control of immune responses.* Eur J Immunol, 2008. **38**(11): p. 2938-42.

48. Marie, C., et al., *Regulation by anti-inflammatory cytokines (IL-4, IL-10, IL-13, TGFbeta)of interleukin-8 production by LPS- and/ or TNFalpha-activated human polymorphonuclear cells.* Mediators Inflamm, 1996. **5**(5): p. 334-40.

49. Palacin, M., et al., *FK506 affects mitochondrial protein synthesis and oxygen consumption in human cells.* Cell Biol Toxicol, 2013. **29**(6): p. 407-14.

50. Wu, D., et al., *Eosinophils sustain adipose alternatively activated macrophages associated with glucose homeostasis.* Science, 2011. **332**(6026): p. 243-7.

51. Molofsky, A.B., et al., *Innate lymphoid type 2 cells sustain visceral adipose tissue eosinophils and alternatively activated macrophages.* J Exp Med, 2013. **210**(3): p. 535-49.

52. Howell, J., et al., *Cyclosporine and tacrolimus have inhibitory effects on toll-like receptor signalling after liver transplantation.* Liver Transplantation, 2013: p. n/a-n/a.

53. Chakkera, H.A., Y. Kudva, and B. Kaplan, *Calcineurin Inhibitors: Pharmacologic Mechanisms Impacting Both Insulin Resistance and Insulin Secretion Leading to Glucose Dysregulation and Diabetes Mellitus.* Clinical Pharmacology & Therapeutics, 2017. **101**(1): p. 114-120.

54. Ekberg, H., et al., *Reduced exposure to calcineurin inhibitors in renal transplantation.* N Engl J Med, 2007. **357**(25): p. 2562-75.

55. Neal, D., *Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids, and weight.* Liver Transplantation, 2001. **7**(6): p. 533-539.

56. Richards, J., et al., *Weight gain and obesity after liver transplantation.* Transplant International, 2005. **18**(4): p. 461-466.
57. Kosteli, A., et al., *Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue.* J Clin Invest, 2010. **120**(10): p. 3466-79.

58. Lacerda, D.R.C., K.A.; Silveira, A.L.M.S.; Rodrigues, D.F.; Silva, A.N.; Sabino, J.L.; Pinho, V.; Menezes, G.B.; Soares, D.D.; Teixeira, M.M.; Ferreira, A.V.M. , *Role of adipose tissue inflammation in fat pad loss induced by fasting in lean and obese mice*. Under review for publication in The Journal of Nutritional Biochemistry.

59. Garcia, M.C., et al., *Mature-onset obesity in interleukin-1 receptor I knockout mice.* Diabetes, 2006. **55**(5): p. 1205-13.

60. Greenberg, A.S., et al., *The role of lipid droplets in metabolic disease in rodents and humans.* J Clin Invest, 2011. **121**(6): p. 2102-10.

61. Clement, K., et al., *Weight loss regulates inflammation-related genes in white adipose tissue of obese subjects.* FASEB J, 2004. **18**(14): p. 1657-69.

62. Jung, S.H., et al., *Effect of weight loss on some serum cytokines in human obesity: increase in IL-10 after weight loss.* J Nutr Biochem, 2008. **19**(6): p. 371-5.

63. Kumagai, H., et al., *Which cytokine is the most related to weight loss-induced decrease in arterial stiffness in overweight and obese men?* Endocr J, 2018. **65**(1): p. 53-61.

64. Lemstra, M., et al., *Weight loss intervention adherence and factors promoting adherence: a meta-analysis.* Patient Prefer Adherence, 2016. **10**: p. 1547-59.

65. Nemes, M.I., et al., *Assessing patient adherence to chronic diseases treatment: differentiating between epidemiological and clinical approaches.* Cad Saude Publica, 2009. **25 Suppl 3**: p. S392-400.

66. Lichtman, S.W., et al., *Discrepancy between self-reported and actual caloric intake and exercise in obese subjects.* N Engl J Med, 1992. **327**(27): p. 1893-8.

67. Ferreira, L.G., et al., *Resting Energy Expenditure, Body Composition, and Dietary Intake.* Transplantation Journal, 2013. **96**(6): p. 579-585.

68. Ribeiro, H.S., et al., *Energy expenditure and balance among long term liver recipients.* Clinical Nutrition, 2014. **33**(6): p. 1147-1152.

69. Yang, Y.J., et al., *Relative validities of 3-day food records and the food frequency questionnaire.* Nutr Res Pract, 2010. **4**(2): p. 142-8.

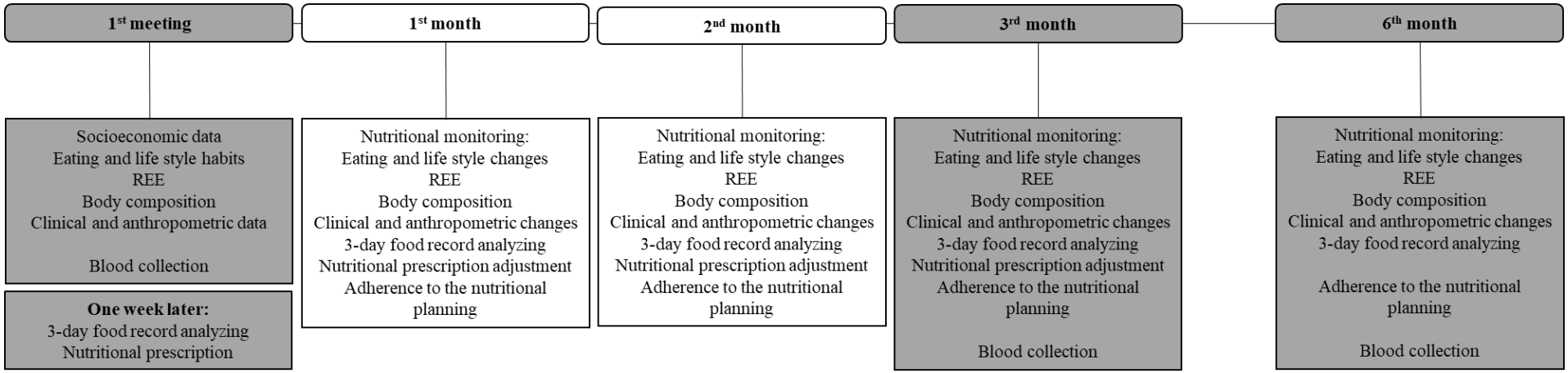

Figure 1: Study design.

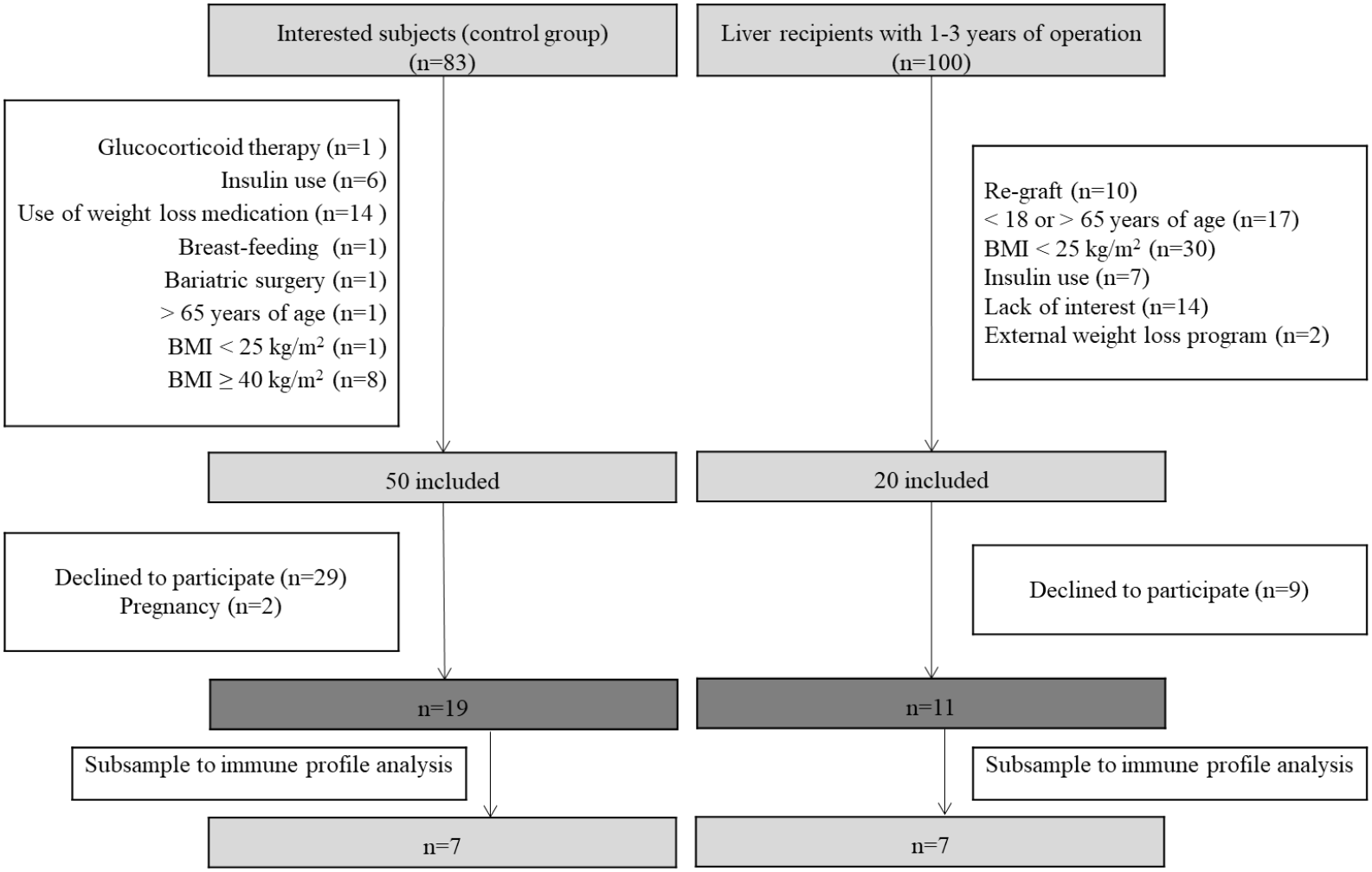

Figure 2: Flow diagram of the study.

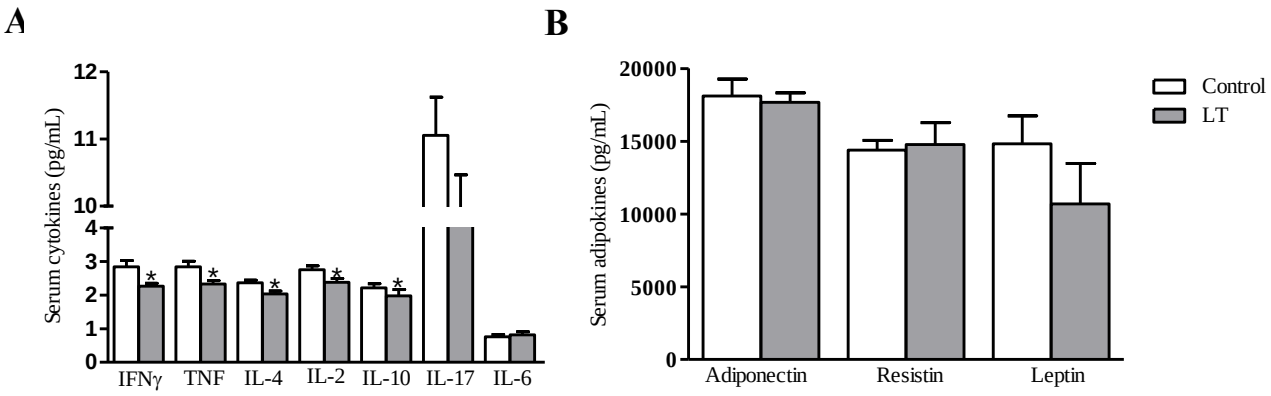

Figure 3: Cytokines serum concentrations in the baseline. * $P < 0,05$ (Student's T-test).

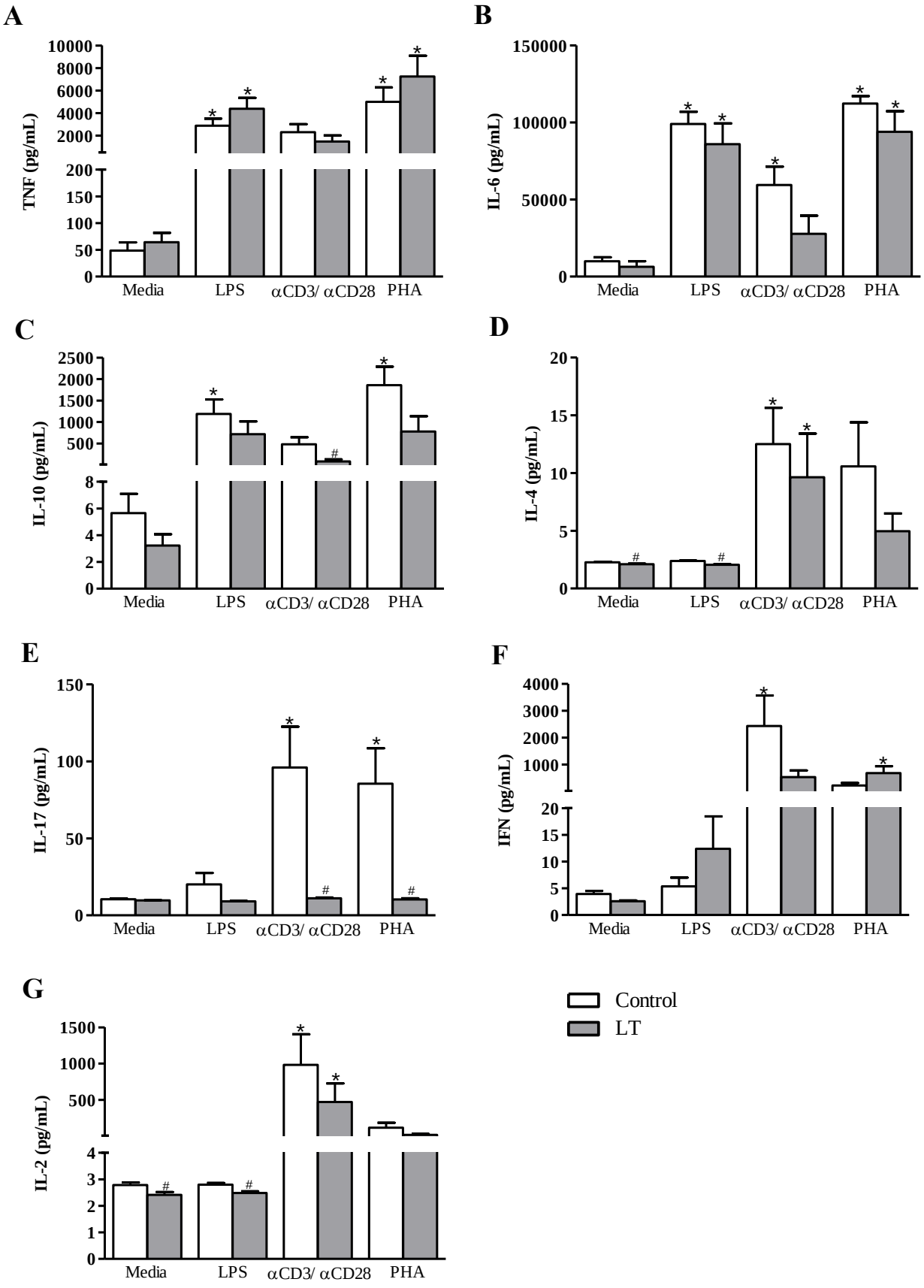

Figure 4: Peripheral blood mononuclear cells (PBMC) culture. PBMC were incubated under different stimuli: lipopolysaccharides (LPS) from *Escherichia coli*, anti-CD3/ anti-CD28, and lectin from *Phaseolus vulgaris* (PHA), $^{\#}$ $P < 0.05$ *versus* the control group at the same time point. * $P < 0.05$ *versus* baseline. Student's T-test.

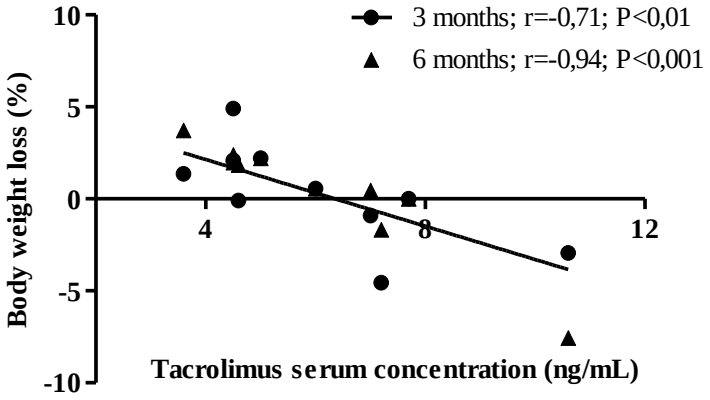

Figure 5: Spearman correlation between body weight loss percentage and tacrolimus serum concentration in the liver recipients.

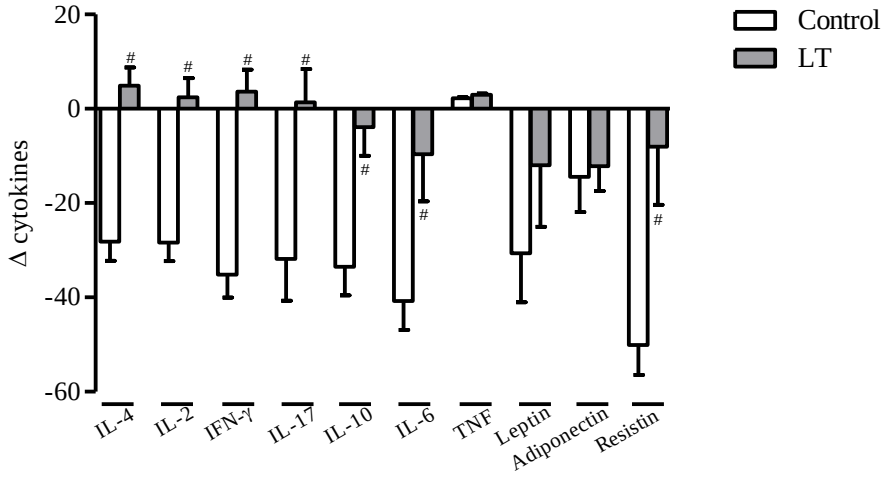

Figure 6: Serum cytokines variation (Δ) after 6 months of follow-up. $^{\#}$ $P < 0.05$ *versus* the control group. Student's T-test.

TABLE 1. Baseline clinical and socioeconomic data of the subjects enrolled in the study

Variables	Control (n=19)	LT (n=11)
Gender		
Male [n (%)]	11 (57.9%)	9 (81.8%)
Female [n (%)]	8 (42.1%)	2 (18.2%)
Age (mean ± S.E.M.)	42 ± 1.9	49 ± 3.6
Marital Status		
Single [n (%)]	2 (10.5%)	1 (9.1%)
Married [n (%)]	16 (84.2%)	8 (72.7%)
Divorced [n (%)]	1 (5.3%)	2 (18.2%)
Family income [$ (min - max)]	1,019.00 (484.00-3,821.00)	922.00 (318.00-4,450.00)
Physical activity level		
Sedentary [n (%)]	11 (57.9%)	7 (63.6%)
Limited activity [n (%)]	5 (26.3%)	3 (27.3%)
Active [n (%)]	3 (15.8)	1 (9.1%)
BMI (kg/m²) (mean ± S.E.M.)	31.0 ± 0.75	28.9 ± 0.98
REE (kcal) (mean ± S.E.M.)	1,877.00 ± 98.8	1,527.18 ± 122.3*
Time since LT (months) (mean ± S.E.M.)	-	23 ± 3
Etiology of liver disease		
Alcohol abuse [n (%)]	-	5 (45.5%)
Hepatitis C virus [n (%)]	-	4 (27.2%)
Cryptogenic cirrhosis [n (%)]	-	2 (18.2%)
Autoimmune hepatitis [n (%)]	-	1 (9.1%)
Immunosuppressive treatment		
Tacrolimus [n (%)]	-	11 (100.0%)
Corticosteroids [n (%)]	-	1 (9,1%)

* $P < 0,05$ (Student's T-test). BMI: body mass index; LT: liver transplantation; REE: resting energy expenditure.

TABLE 2. Immune cell profile of control and LT subjects

Frequency [% (min - max)]	Control (n=7)	LT (n=7)
LYMPHOCYTES		
Total CD3+	70.3 (65.5 - 82.4)	71.0 (51.3 - 80.4)
T helper Cells		
CD3+CD4+	64.3 (39.2 - 76.1)	54.5 (37.4 - 79.2)
CD3+CD4+CD69+	94.8 (92.8 - 95.8)	96.8 (95.0 - 98.4)
CD3+CD4+CD25^{low}	6.8 (4.0 - 10.1)	5.9 (3.5 - 17.6)
CD3+CD4+IFN+	1.1 (0.8 - 1.9)	1.21 (0.9 - 1.9)
CD3+CD4+IL1β+	0.2 (0.2 - 0.3)	0.4 (0.2 - 0.4)
CD3+CD4+IL6+	0.4 (0.3 - 0.8)	0.7 (0.2 - 1.5)
CD3+CD4+TNF+	0.3 (0.2 - 0.5)	0,3 (0.2 - 1.2)
Treg cells		
CD3+CD4+CD25^{high}	3.8 (2.3 - 5.2)	2.3 (1.7 - 7.3)
CD3+CD4+CD25^{high}FOXP3+	66.8 (64.4 - 75.5)	68.1 (55.5 - 76.7)
CD3+CD4+CD25^{high}FOXP3+IL10+	1.1 (0.7 - 1.9)	1.4 (0.7 - 2.8)
Cytotoxic T cells		
CD3+CD8^{high}	28.8 (15.9 - 49.1)	43.1 (15.5 - 55.9)
CD3+CD8^{high}CD69+	23.5 (12.5 - 45.0)	22.1 (6.7 - 38.7)
CD3+CD8^{high}CD25+	29.0 (28.5 - 29.9)	30.5 (29.4 - 34.5)
CD3+CD8^{high}IL10+	5.4 (4.0 - 7.2)	6.2 (4.0 - 8.4)
CD3+CD8^{high}IL1β+	3.3 (1.1 - 4.1)	3.6 (2.7 - 4.3)
CD3+CD8^{high}IFN+	4.0 (3.0 - 7.0)	3.4 (1.9 - 4.8)
CD3+CD8^{high}TNF+	2.9 (1.0 - 3.2)	3.9 (2.5 - 5.1)
CD3+CD8^{high}IL6+	3.3 (1.3 -5.0)	3.3 (3.1 - 4.7)
B lymphocytes		
CD3-CD19+	29.9 (19.8 - 42.7)	46.2 (25.1 - 54.6)*
CD3-CD19+CD69+	0.9 (0.6 - 1.5)	0.8 (0.4 - 1.2)
NATURAL KILLER (NK)		
CD8^{low}CD56+	12.0 (3.7 - 16.1)	5.5 (5.1 - 21.3)
CD8^{low}CD56+CD16+	94.0 (88.8 - 98.3)	85.2 (61.8 - 95.6)*
CD8^{low}CD56+CD16+IFN+	2.1 (0.7 - 4.3)	1.5 (1.3 - 3.1)
CD8^{low}CD56+CD16+IL1β+	4.1 (2.0 - 7.5)	5.1 (3.0 - 7.3)
CD8^{low}CD56+CD16+IL6+	4.4 (2.4 - 8.0)	6.2 (3.1 - 6.8)
CD8^{low}CD56+CD16+TNF+	2.8 (1.6 - 4.7)	3.3 (2.1 - 9.2)
MONOCYTES		
Total CD14+	54.2 (39.4 - 84.0)	67.0 (50.4 - 79.8)
CD14+CD16+	24.1 (13.9 - 41.0)	25.8 (23.3 - 36.7)
CD14+CD16-	25.8 (13.2 - 59.9)	37.2 (26.2 - 48.6)
CD14+CD16+IL1β+	12.7 (8.4 - 32.0)	18.2 (9.0 - 23.3)
CD14+CD16-IL1β+	6.3 (2.1 - 7.7)	6.9 (2.8 - 13.8)
CD14+CD16+IL6+	14.9 (7.9 - 40.3)	17.0 (12.7 - 25.0)
CD14+CD16-IL6+	8.4 (3.5 - 15.3)	10.3 (5.7 - 16.1)
CD14+CD16+TNF+	7.9 (4.3 - 30.2)	12.9 (9.1 - 20.4)
CD14+CD16-TNF+	5.0 (1.1 - 9.4)	5.6 (3.7 - 11.5)

* $P < 0,05$ (Mann-Whitney test). LT: liver transplantation.

TABLE 3. Adherence to the nutritional counselling

Level of adherence	3 months		6 months	
	Control (n=19)	LT (n=11)	Control (n=19)	LT (n=11)
Adhered to all orientations received	3 (15.8%)	3 (27.2%)	4 (21.1%)	2 (18.2%)
Adhered to the orientations for some time	7 (36.8%)	1 (9.1%)	4 (21.1%)	-
Adhered only to some orientations received	5 (26.3%)	5 (45.5%)	7 (36.7%)	5 (45.5%)
Tried to adhere, but it was unsuccessful	3 (15.8%)	2 (18.2%)	4 (21.1%)	4 (36.3%)
Did not try to follow any orientation	1 (5.3%)	-	-	-

Chi-square test. LT: liver transplantation

TABLE 4. Changes in body composition of participants throughout the study

Variables (mean ± S.E.M.)	Control (n=19)			Liver transplantation (n=11)		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Body weight loss (%)	-	1.77 ± 1.33	3.73 ± 1.09	-	0.42 ± 0.47[#]	0.54 ± 0.92[#]
Body weight (kg)	89.74 ± 3,69	88.68 ± 4.56	86.63 ± 3.86*	80.18 ± 3.83	80.01 ± 3.72	79.73 ± 3.64
Body fat mass (kg)	31.10 ± 0.70	30.10 ± 0.84*	30.00 ± 0.87*	28.91 ± 0.96	28.77 ± 0.87	28.64 ± 0.79
Fat-free mass (kg)	57.58 ± 3.27	56.47 ± 3.17	56.51 ± 3.14	52.18 ± 2.04	51.70 ± 2.19	51.45 ± 2.28
Waist circumference (cm)	103.84 ± 2.36	101.37 ± 2.60*	101.31 ± 2.50*	103.73 ± 2.13	102.50 ± 2.31	102.27 ± 2.03

[#] $P < 0.05$ versus the control group at the same time point. * $P < 0.05$ versus baseline. GEE model.

TABLE 5. Energy and macronutrient intake of participants throughout the study

Variables (mean ± S.E.M.)	Control (n=19)			Liver transplantation (n=11)		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Energy intake (kcal)	2097.27 ± 77.98	1843.45 ± 118.12	1833.12 ± 172.27	2094.00 ± 232.04	1977.03 ± 207.20	1940.92 ± 106.48
Carbohydrates (g)	257.22 ± 9.97	212.17 ± 11.45	241.06 ± 22.08	267.50 ± 28.44	259.14 ± 24.99	225.67 ± 26.26
Proteins (g)	94.78 ± 5.60	92.56 ± 8.02	77.35 ± 7.66	84.67 ± 10.81	84.71 ± 8.89	112.23 ± 21.62
Lipids (g)	77.28 ± 4.19	71.08 ± 6.45	64.47 ± 7.69	77.33 ± 9.91	68.74 ± 10.32	87.72 ± 17.10

GEE model.

TABLE 6. Metabolites serum concentrations of the volunteers in the follow-up period

Variables (mean ± S.E.M.)	Control (n=19)			Liver transplantation (n=11)		
	Baseline	3 months	6 months	Baseline	3 months	6 months
Glucose (mg/dL)	87.2 ± 3.8	87.5 ± 2.6	97.9 ± 7.5	87.5 ± 3.2	91.3 ± 2.8	90.4 ± 1.6
Triglycerides (mg/dL)	170.1 ± 21.7	183.6 ± 25.8	176.6 ± 23.5	144.2 ± 18.6	152.1 ± 11.1	153.6 ± 11.3
Total cholesterol (mg/dL)	180.0 ± 12.1	182.3 ± 14.1	171.6 ±12.3	123.9 ± 6.4[#]	143.6 ± 13.6[#]	144.4 ± 10.0
LDL cholesterol (mg/dL)	93.8 ± 9.4	106.0 ± 14.9	98.5 ± 12.6	65.2 ± 8.6[#]	73.4 ± 11.5	63.7 ± 10.5
HDL cholesterol (mg/dL)	42.2 ± 5.1	34.4 ± 2.3	42.3 ± 2.9	33.4 ± 3.1	36.8 ± 3.3	40.5 ± 3.3
VLDL cholesterol (mg/dL)	34.7 ± 4.3	37.8 ± 4.2	36.7 ± 4.9	39.9 ± 8.2	42.2 ± 4.5	44.9 ± 7.4

[#] $P < 0.05$ versus the control group at the same time point. GEE model.

6. CONCLUSÃO

Este trabalho apresenta dois fatos importantes sobre a população estudada. A princípio, demonstrou-se que pacientes transplantados hepáticos com sobrepeso/ obesidade são hipometabólicos, o que representa um importante fator de risco para o desenvolvimento de obesidade nessa população. No entanto, quando esses pacientes recebem um planejamento alimentar com foco na perda de peso, tal objetivo não é alcançado como ocorre em pacientes não-transplantados. Essa ausência de resposta à intervenção dietética foi relacionada ao comprometimento do sistema imunológico desses pacientes, que apresentaram menor responsividade inflamatória após diferentes estímulos, quando comparados a indivíduos não-imunossuprimidos e não-transplantados. Além disso, foram observadas baixas concentrações séricas de citocinas, como IFN-γ, TNF, IL-4, IL-2 e IL-10, apesar de apresentarem frequência normal de células mononucleares periféricas, com exceção de células NK $CD8^{low}CD56^{+}CD16^{+}$ e linfócitos B.

Esses dados contribuem para o entendimento da estreita associação entre obesidade e resposta inflamatória, ratificando a hipótese de que a inflamação pode exercer papel importante e fisiológico no controle do peso corporal.

7. REFERÊNCIAS (INTRODUÇÃO E REVISÃO DE LITERATURA)

ABDULRAHMAN, M. M. et al. New-onset diabetes after transplantation among renal transplant recipients at a new transplant center; King Fahad Specialist Hospital-Dammam, Saudi Arabia. **Saudi J Kidney Dis Transpl,** v. 29, n. 4, p. 863-871, Jul-Aug 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30152423 >.

AEBERLI, I. et al. Low to moderate sugar-sweetened beverage consumption impairs glucose and lipid metabolism and promotes inflammation in healthy young men: a randomized controlled trial. **Am J Clin Nutr,** v. 94, n. 2, p. 479-85, Aug 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21677052 >.

AHLSTROM, P. et al. Adiponectin improves insulin sensitivity via activation of autophagic flux. **J Mol Endocrinol,** v. 59, n. 4, p. 339-350, Nov 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28954814 >.

ALI, A. et al. Association of Obesity and Thyroid Cancer at a Tertiary Care Hospital in Pakistan. **Cureus,** v. 10, n. 3, p. e2364, Mar 26 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29805933 >.

ANASTACIO, L. R. et al. Body composition and overweight of liver transplant recipients. **Transplantation,** v. 92, n. 8, p. 947-51, Oct 27 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21869739 >.

ANASTACIO, L. R. et al. Overweight, obesity and weight gain up to three years after liver transplantation. **Nutr Hosp,** v. 27, n. 4, p. 1351-6, Jul-Aug 2012 Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23165585 >.

ANASTACIO, L. R. et al. Overweight in liver transplant recipients. **Rev Col Bras Cir,** v. 40, n. 6, p. 502-7, Nov-Dec 2013. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24573630 >.

ANDRADE, A. R. et al. New Onset Diabetes and Non-Alcoholic Fatty Liver Disease after Liver Transplantation. **Ann Hepatol,** v. 16, n. 6, p. 932-940, November-December 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29055928 >.

AZZI, J. R.; SAYEGH, M. H.; MALLAT, S. G. Calcineurin inhibitors: 40 years later, can't live without. **J Immunol,** v. 191, n. 12, p. 5785-91, Dec 15 2013. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24319282 >.

BABU, G. R. et al. Association of obesity with hypertension and type 2 diabetes mellitus in India: A meta-analysis of observational studies. **World J Diabetes,** v. 9, n. 1, p. 40-52, Jan 15 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29359028 >.

BIANCHI, G. et al. Metabolic syndrome in liver transplantation: relation to etiology and immunosuppression. **Liver Transpl,** v. 14, n. 11, p. 1648-54, Nov 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18975273 >.

BRAM, R. J. et al. Identification of the immunophilins capable of mediating inhibition of signal transduction by cyclosporin A and FK506: roles of calcineurin binding and cellular location. **Mol Cell Biol,** v. 13, n. 8, p. 4760-9, Aug 1993. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7687744 >.

BRASIL. Ministério da Saúde. Secretaria de Vigilância em Saúde. VIGITEL Brasil 2016: Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico. p. 160, 2017.

BROWN, R. A. et al. Long-term effects of anti-tumour necrosis factor therapy on weight in patients with rheumatoid arthritis. **Clin Rheumatol,** v. 31, n. 3, p. 455-61, Mar 2012. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22009196 >.

BULUM, B. et al. Hypertension in children after renal transplantation. **Pediatr Int,** v. 57, n. 6, p. 1138-42, Dec 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26009796 >.

CARR, W. W. Topical calcineurin inhibitors for atopic dermatitis: review and treatment recommendations. **Paediatr Drugs,** v. 15, n. 4, p. 303-10, Aug 2013. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23549982 >.

CHANG, K. T. et al. Tacrolimus suppresses atopic dermatitis-associated cytokines and chemokines in monocytes. **J Microbiol Immunol Infect,** v. 49, n. 3, p. 409-16, Jun 2016. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25315214 >.

CHANG, S. H.; MCDONALD, S. P. Post-kidney transplant weight change as marker of poor survival outcomes. **Transplantation,** v. 85, n. 10, p. 1443-8, May 27 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18497685 >.

CHARLTON, M. et al. Everolimus Is Associated With Less Weight Gain Than Tacrolimus 2 Years After Liver Transplantation: Results of a Randomized Multicenter Study. **Transplantation,** v. 101, n. 12, p. 2873-2882, Dec 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28817434 >.

CHEN, Y. et al. Changes in Resting Energy Expenditure Following Orthotopic Liver Transplantation. **JPEN J Parenter Enteral Nutr,** v. 40, n. 6, p. 877-82, Aug 2016. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25940610 >.

CHOE, S. S. et al. Adipose Tissue Remodeling: Its Role in Energy Metabolism and Metabolic Disorders. **Front Endocrinol (Lausanne),** v. 7, p. 30, 2016. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27148161 >.

CHYLIKOVA, J. et al. M1/M2 macrophage polarization in human obese adipose tissue. **Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub,** v. 162, n. 2, p. 79-82, Jun 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29765169 >.

CONZEN, K. D. et al. Morbid obesity in liver transplant recipients adversely affects longterm graft and patient survival in a single-institution analysis. **HPB (Oxford),** v. 17, n. 3, p. 251-7, Mar 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25322849 >.
CORVERA, S.; GEALEKMAN, O. Adipose tissue angiogenesis: impact on obesity and type-2 diabetes. **Biochim Biophys Acta,** v. 1842, n. 3, p. 463-72, Mar 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23770388 >.

DALLMAN, M. F. et al. Minireview: glucocorticoids--food intake, abdominal obesity, and wealthy nations in 2004. **Endocrinology,** v. 145, n. 6, p. 2633-8, Jun 2004. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15044359 >.

DUCLOUX, D. et al. One-year post-transplant weight gain is a risk factor for graft loss. **Am J Transplant,** v. 5, n. 12, p. 2922-8, Dec 2005. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16303006 >.

DUFFAUT, C. et al. Unexpected trafficking of immune cells within the adipose tissue during the onset of obesity. **Biochem Biophys Res Commun,** v. 384, n. 4, p. 482-5. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19422792 >.

DUTTA, S.; AHMAD, Y. The efficacy and safety of tacrolimus in rheumatoid arthritis. **Ther Adv Musculoskelet Dis,** v. 3, n. 6, p. 283-91, Dec 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22870486 >.

FERREIRA, L. G. et al. Resting energy expenditure, body composition, and dietary intake: a longitudinal study before and after liver transplantation. **Transplantation,** v. 96, n. 6, p. 579-85, Sep 2013. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/23851933 >.

FERREIRA, L. G. et al. Resting Energy Expenditure, Body Composition, and Dietary Intake. **Transplantation Journal,** v. 96, n. 6, p. 579-585, 2013.

FUSSNER, L. A. et al. Cardiovascular disease after liver transplantation: When, What, and Who Is at Risk. **Liver Transpl,** v. 21, n. 7, p. 889-96, Jul 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25880971 >.

GREENBERG, A. S.; OBIN, M. S. Obesity and the role of adipose tissue in inflammation and metabolism. **Am J Clin Nutr,** v. 83, n. 2, p. 461S-465S, Feb 2006. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16470013 >.

GREGOR, M. F.; HOTAMISLIGIL, G. S. Inflammatory mechanisms in obesity. **Annu Rev Immunol,** v. 29, p. 415-45, 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21219177 >.

HENG, A. E. et al. Energy expenditure, spontaneous physical activity and with weight gain in kidney transplant recipients. **Clin Nutr,** v. 34, n. 3, p. 457-64, Jun 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24928604 >.

HOTAMISLIGIL, G. S. Inflammation and metabolic disorders. **Nature,** v. 444, n. 7121, p. 860-7, Dec 14 2006. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17167474 >.

HOTAMISLIGIL, G. S.; SHARGILL, N. S.; SPIEGELMAN, B. M. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. **Science,** v. 259, n. 5091, p. 87-91, Jan 1 1993. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/7678183.

KOSTELI, A. et al. Weight loss and lipolysis promote a dynamic immune response in murine adipose tissue. **J Clin Invest,** v. 120, n. 10, p. 3466-79, Oct 2010. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/20877011 >.

KOTSIS, V. et al. Obesity and cardiovascular risk: a call for action from the European Society of Hypertension Working Group of Obesity, Diabetes and the High-risk Patient and European Association for the Study of Obesity: part A: mechanisms of obesity induced hypertension, diabetes and dyslipidemia and practice guidelines for treatment. **J Hypertens,** v. 36, n. 7, p. 1427-1440, Jul 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29634663 >.

KUGLER, C. et al. Postoperative weight gain during the first year after kidney, liver, heart, and lung transplant: a prospective study. **Prog Transplant,** v. 25, n. 1, p. 49-55, Mar 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25758801 >.

KUO, F. C. et al. Circulating Soluble IL-6 Receptor Concentration and Visceral Adipocyte Size Are Related to Insulin Resistance in Taiwanese Adults with Morbid Obesity. **Metab Syndr Relat Disord,** v. 15, n. 4, p. 187-193, May 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28346858 >.

LARSEN, G. L.; HENSON, P. M. Mediators of inflammation. **Annu Rev Immunol,** v. 1, p. 335-59, 1983. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/6399978 >.

LAURES, A. S. et al. Risk factors for cardiovascular disease during the first 2 years after renal transplantation. **Transplant Proc,** v. 37, n. 9, p. 3778-81, Nov 2005. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16386536 >.

LI, W. et al. TNF-alpha stimulates endothelial palmitic acid transcytosis and promotes insulin resistance. **Sci Rep,** v. 7, p. 44659, Mar 17 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28304381 >.

LIN, Y. et al. Association between obesity and bladder cancer recurrence: A meta-analysis. **Clin Chim Acta,** v. 480, p. 41-46, May 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29408169 >.

LOPEZ-GARCIA, E. et al. Consumption of trans fatty acids is related to plasma biomarkers of inflammation and endothelial dysfunction. **J Nutr,** v. 135, n. 3, p. 562-6, Mar 2005 Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15735094 >.

LOPEZ-VILELLA, R. et al. Incidence of development of obesity after heart transplantation according to the calcineurin inhibitor. **Transplant Proc,** v. 47, n. 1, p. 127-9, Jan-Feb 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25645789 >.

MAKKI, K.; FROGUEL, P.; WOLOWCZUK, I. Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. **ISRN Inflamm,** v.

2013, p. 139239, Dec 22 2013. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24455420 >.

MARTINS, L. B. et al. Paradoxical role of Tumor Necrosis Factor on metabolic dysfunction and adipose tissue expansion in mice. **Nutrition**, 2017.

MARTINS, L. B. et al. Paradoxical role of tumor necrosis factor on metabolic dysfunction and adipose tissue expansion in mice. **Nutrition,** v. 50, p. 1-7, Jun 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/29510322 >.

MEDZHITOV, R. Origin and physiological roles of inflammation. **Nature,** v. 454, n. 7203, p. 428-35, Jul 24 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18650913 >.

MENEZES-GARCIA, Z. et al. Lack of platelet-activating factor receptor protects mice against diet-induced adipose inflammation and insulin-resistance despite fat pad expansion. **Obesity (Silver Spring),** v. 22, n. 3, p. 663-72, Mar 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24339378 >.

NDISANG, J. F.; VANNACCI, A.; RASTOGI, S. Oxidative stress and inflammation in obesity, diabetes, hypertension, and related cardiometabolic complications. **Oxid Med Cell Longev,** v. 2014, p. 506948, 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24723993 >.

NEAL, D. A. et al. Beneficial effects of converting liver transplant recipients from cyclosporine to tacrolimus on blood pressure, serum lipids, and weight. **Liver Transpl,** v. 7, n. 6, p. 533-9, Jun 2001. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11443583 >.

OUCHI, N. et al. Adipokines in inflammation and metabolic disease. **Nat Rev Immunol,** v. 11, n. 2, p. 85-97, Feb 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21252989

PARMENTIER-DECRUCQ, E. et al. Effects of infliximab therapy on abdominal fat and metabolic profile in patients with Crohn's disease. **Inflamm Bowel Dis,** v. 15, n. 10, p. 1476-84, Oct 2009. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19291781 >.

PERSEGHIN, G. et al. Resting energy expenditure in diabetic and nondiabetic patients with liver cirrhosis: relation with insulin sensitivity and effect of liver transplantation and immunosuppressive therapy. **Am J Clin Nutr,** v. 76, n. 3, p. 541-8, Sep 2002. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12197997 >.

RANA, J. S. et al. Cardiovascular metabolic syndrome - an interplay of, obesity, inflammation, diabetes and coronary heart disease. **Diabetes Obes Metab,** v. 9, n. 3, p. 218-32, May 2007. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/17391148 >.

RAO, A.; LUO, C.; HOGAN, P. G. Transcription factors of the NFAT family: regulation and function. **Annu Rev Immunol,** v. 15, p. 707-47, 1997. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/9143705 >.

RENZO, L. D. et al. Prospective assessment of body weight and body composition changes in patients with psoriasis receiving anti-TNF-alpha treatment. **Dermatol Ther,** v. 24, n. 4, p. 446-51, Jul-Aug 2011. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/21910803 >.

RIBEIRO, H. S. et al. Prevalence and factors associated with dyslipidemia after liver transplantation. **Rev Assoc Med Bras (1992),** v. 60, n. 4, p. 365-72, Jul 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25211421 >.

RIBEIRO, H. S. et al. Energy expenditure and balance among long term liver recipients. **Clin Nutr,** v. 33, n. 6, p. 1147-52, Dec 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24423749 >.

RIBEIRO, H. S. et al. Energy expenditure and balance among long term liver recipients. **Clinical Nutrition,** v. 33, n. 6, p. 1147-1152, 2014.

RICHARDS, J. et al. Weight gain and obesity after liver transplantation. **Transpl Int,** v. 18, n. 4, p. 461-6, Apr 2005. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/15773968 >.

RICHARDS, J. et al. Weight gain and obesity after liver transplantation. **Transplant International,** v. 18, n. 4, p. 461-466, 2005.

RICHARDSON, R. A.; GARDEN, O. J.; DAVIDSON, H. I. Reduction in energy expenditure after liver transplantation. **Nutrition,** v. 17, n. 7-8, p. 585-9, Jul-Aug 2001. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/11448577 >.

RINK, J. D. et al. Cellular characterization of adipose tissue from various body sites of women. **J Clin Endocrinol Metab,** v. 81, n. 7, p. 2443-7, Jul 1996. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8675558 >.

RODRIGUES, D. F. et al. Acute intake of a high-fructose diet alters the balance of adipokine concentrations and induces neutrophil influx in the liver. **J Nutr Biochem,** v. 25, n. 4, p. 388-94, Apr 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24485988 >.

ROGERS, C. C. et al. Body weight alterations under early corticosteroid withdrawal and chronic corticosteroid therapy with modern immunosuppression. **Transplantation,** v. 80, n. 1, p. 26-33, Jul 15 2005. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/16003229 >.

ROSENBAUM, M.; LEIBEL, R. L. 20 years of leptin: role of leptin in energy homeostasis in humans. **J Endocrinol,** v. 223, n. 1, p. T83-96, Oct 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/25063755 >.

SARACENO, R. et al. Effect of anti-tumor necrosis factor-alpha therapies on body mass index in patients with psoriasis. **Pharmacol Res,** v. 57, n. 4, p. 290-5, Apr 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18400510 >.

SARWAR, R.; PIERCE, N.; KOPPE, S. Obesity and nonalcoholic fatty liver disease: current perspectives. **Diabetes Metab Syndr Obes,** v. 11, p. 533-542, 2018. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/30288073 >.

SCHUTZ, T. et al. Weight gain in long-term survivors of kidney or liver transplantation--another paradigm of sarcopenic obesity? **Nutrition,** v. 28, n. 4, p. 378-83, Apr 2012. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/22304858 >.

SHIBASAKI, F. et al. Role of kinases and the phosphatase calcineurin in the nuclear shuttling of transcription factor NF-AT4. **Nature,** v. 382, n. 6589, p. 370-3, Jul 25 1996. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/8684469 >.

SINDHU, S. et al. Obesity Is a Positive Modulator of IL-6R and IL-6 Expression in the Subcutaneous Adipose Tissue: Significance for Metabolic Inflammation. **PLoS One,** v. 10, n. 7, 2015. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/26200663 >.

SONG, X. et al. Changes in pro-inflammatory cytokines and body weight during 6-month risperidone treatment in drug naive, first-episode schizophrenia. **Psychopharmacology (Berl),** v. 231, n. 2, p. 319-25, Jan 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24337064 >.

VERMA, K. K. et al. Azathioprine versus betamethasone for the treatment of parthenium dermatitis: a randomized controlled study. **Indian J Dermatol Venereol Leprol,** v. 74, n. 5, p. 453-7, Sep-Oct 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/19052402 >.

VIRDIS, A. Endothelial Dysfunction in Obesity: Role of Inflammation. **High Blood Press Cardiovasc Prev,** v. 23, n. 2, p. 83-5, Jun 2016. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/27000854 >.

WEGHUBER, D. et al. Impact of age and metabolic syndrome on the adipokine profile in childhood and adult obesity. **Exp Clin Endocrinol Diabetes,** v. 122, n. 6, p. 363-7, Jun 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24941433 >.

WERNSTEDT ASTERHOLM, I. et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. **Cell Metab,** v. 20, n. 1, p. 103-18, Jul 1 2014. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/24930973 >.

WHO. World Health Organization. Obesity and overweight. 2016. Disponível em: < http://www.who.int/en/news-room/fact-sheets/detail/obesity-and-overweight.

WU, H.; BALLANTYNE, C. M. Skeletal muscle inflammation and insulin resistance in obesity. **J Clin Invest,** v. 127, n. 1, p. 43-54, Jan 3 2017. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/28045398 >.

ZHANG, C. The role of inflammatory cytokines in endothelial dysfunction. **Basic Res Cardiol,** v. 103, n. 5, p. 398-406, Sep 2008. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/18600364 >.

ZHANG, H. H. et al. Tumor necrosis factor-alpha stimulates lipolysis in differentiated human adipocytes through activation of extracellular signal-related kinase and elevation of intracellular cAMP. **Diabetes,** v. 51, n. 10, p. 2929-35, Oct 2002. Disponível em: < http://www.ncbi.nlm.nih.gov/pubmed/12351429 >.