UNIVERSIDADE ESTADUAL DE CAMPINAS
Faculdade de Engenharia de Alimentos

NATHALIA MEDINA DOS SANTOS

IMPACTO DO CONSUMO DE POLPA DE AÇAÍ PARA A PREVENÇÃO DO COMPROMETIMENTO COGNITIVO

IMPACT OF AÇAI PULP CONSUMPTION FOR THE PREVENTION OF COGNITIVE IMPAIRMENT

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Dissertação apresentada à Faculdade de Engenharia de Alimentos da Universidade Estadual de Campinas como parte dos requisitos exigidos para a obtenção do título de Mestra em Alimentos e Nutrição, na Área de Nutrição Experimental e aplicada à Tecnologia de Alimentos.

Dissertation presented to the Faculty of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Master in Food and Nutrition the area of Experimental Nutrition and Nutrition applied to Food Technology.

Orientador: Prof. Dr. Mário Roberto Maróstica Júnior

ESTE EXEMPLAR CORRESPONDE À VERSÃO FINAL DEFENDIDA PELA ALUNA NATHALIA MEDINA DOS SANTOS E ORIENTADA PELO PROF. DR. MÁRIO ROBERTO MARÓSTICA JÚNIOR

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A ata de defesa, com as respectivas assinaturas dos membros, encontra-se no processo de vida acadêmica do aluno.

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de acreditar e lutar pelos seus sonhos.

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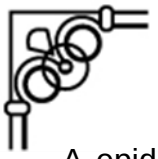


RESUMO

A epidemia da obesidade vem se tornando cada vez mais um problema de saúde pública em todo o mundo. Além das consequências à saúde já muito bem fundamentadas, como as doenças cardiovasculares, alguns tipos de câncer, síndrome metabólica, diabetes do tipo 2, mais recentemente, também tem sido associada à insuficiências cognitivas e demência. O consumo de alimentos naturais seria alternativa para minimizar e/ou reverter os efeitos da dieta contemporânea, rica em açúcares simples e gordura saturada, que acompanha o ganho de peso da população mundial. O açaí é um fruto tipicamente brasileiro que ganhou muita atenção devido ao seu alto potencial antioxidante. O fruto conta com grande quantidade de compostos bioativos de interesse. Assim, nosso foi investigar estes compostos e seus efeitos na prevenção da obesidade e doenças associadas, como a resistência periférica à insulina, e o desempenho cognitivo em camundongos. Para isto, polpas comerciais de açaí adquiridas em Manaus-AM foram liofilizadas e analisadas quanto à sua composição de macronutrientes, antioxidantes, compostos fenólicos, carotenoides e composição lipídica. A polpa seca foi adicionada à dietas normo e hiperlipídicas e administradas por 10 semanas à camundongos adultos. Na antepenúltima semana de ensaio os animais passaram por um teste de tolerância à glicose, na penúltima por um teste de tolerância à insulina e na última semana de ensaio, por um teste de cognição. Ao final do experimento, os animais foram sacrificados e foram coletados o soro, tecido adiposo e hipocampo e avaliou-se a adiposidade, estresse oxidativo, resistência à insulina periférica e via da sinalização da fosforilação da proteína tau estabilizante de microtúbulo. A dieta hiperlipídica promoveu maior ganho de peso corporal, maior resistência à insulina periférica, diminuiu a atividade de enzimas do metabolismo antioxidante, aumentou a fosforilação da tau no hipocampo e implicou em prejuízo do desempenho cognitivo dos animais. A suplementação com a polpa de açaí promoveu maior sensibilidade periférica à insulina nos animais obesos, menor peso relativo dos tecidos adiposos viscerais, e, apesar de ter sido observada apenas uma tendência de menor fosforilação da tau, estes animais mostraram melhor desempenho no teste de reconhecimento de objeto novo. Em conclusão, a suplementação de dietas com a polpa de açaí preveniu o ganho de gordura visceral, a resistência à insulina e indicou efeito neuroprotetor, minimizando os déficits cognitivos.

ABSTRACT

The obesity epidemy has increasingly become a public health problem throughout the world. In addition to the already well-founded health consequences such as cardiovascular diseases, cancer, metabolic syndrome and type 2 diabetes and more recently, cognitive impairment and dementia have also been associated with overweight. The consumption of natural foods would be an alternative to minimize and / or reverse the effects of the contemporary diet, rich in simple sugars and saturated fatty acids, which follows the weight gain of the world population. Açai is a Brazilian fruit that has gained a lot of attention due to its high antioxidant potential and large amount of bioactive compounds. Thus, our objective was to investigate these compounds and their effects on the prevention of obesity and associated diseases, such as peripheral insulin resistance and cognitive impairment in mice. For this purpose, commercial açai pulps purchased in Manaus-AM were lyophilized and analyzed regarding its composition of macronutrients, antioxidants, phenolic compounds, carotenoids and fatty acids content. The freezed-dried pulp was added to normal and high-fat diets and administered to adult mice for 10 weeks. In the antepenultimate experimental week, the animals underwent a glucose tolerance test, and in the penultimate week, an insulin tolerance test and in the last week, a cognition test. At the end of the experiment, animals were sacrificed and the serum, adipose tissue and hippocampus were collected for adiposity, oxidative stress, peripheral insulin resistance and phosphorylation of the tau microtubule stabilizing protein evaluation. The high-fat diet was able to promote greater body weight gain, greater resistance to peripheral insulin, decreased enzyme activity of the antioxidant metabolism of the animals, increased tau phosphorylation in the hippocampus, which implied impaired cognitive performance in the test of recognition of new object by the animals. However, supplementation with açai pulp improved peripheral insulin sensitivity in obese animals, lower relative-to-body weight of visceral adipose tissues, and although only a trend of lower phosphorylation of tau was observed, these animals showed better performance in the new object recognition test. In conclusion, dietary supplementation with açai pulp prevented visceral fat gain, peripheral insulin resistance and indicated neuroprotective effect, preventing cognitive impairment.

SUMÁRIO

INTRODUÇÃO GERAL

O sobrepeso e a obesidade são apontados como problemas de saúde pública e corroboram com o aumento na prevalência de doenças crônicas metabólicas e degenerativas em todo o mundo (WHO, 2003). Nos últimos anos, o aumento da proporção de indivíduos com sobrepeso na população tem crescido de forma expressiva nas nações desenvolvidas (BHUROSY; JEEWON, 2014). Este fenômeno tem se replicado nas nações em desenvolvimento, como o Brasil, onde o percentual dos indivíduos considerados obesos vem crescendo nas últimas décadas (FERREIRA; MAGALHÃES, 2006).

De acordo com dados da VIGITEL (Vigilância de Fatores de Risco e Proteção para Doenças Cardiovasculares por Inquérito Telefônico) de 2016, 53,8% dos brasileiros estão acima do peso e quase 19% já tem um quadro de obesidade estabelecido, de acordo com o índice de massa corporal (IMC); o número de obesos no Brasil cresceu 60% em dez anos, passando de 11,8% em 2006 para 18,9% em 2016 e o sobrepeso cresceu 23,6%, passando de 42,6% em 2006 para 53,8% em 2016 (BRASIL, 2017). Estes dados voltam a atenção também para o índice de mortalidade por doenças crônicas não transmissíveis, que são responsáveis por 72% de óbitos no país, sendo a obesidade importante fator de risco de desenvolvimento de doenças como o diabetes (Ministério da Saúde, 2015).

Fatores genéticos, ambientais e psicossociais são determinantes no desenvolvimento da obesidade (KOPELMAN, 2000). No entanto, as mudanças ocorridas no estilo de vida nos últimos anos sugerem que esta condição se deve principalmente ao desequilíbrio energético resultante do aporte calórico da dieta superior às necessidades energéticas individuais, aliado ao sedentarismo (FRANKLIN; KANALEY, 2009). A alimentação de indivíduos obesos é majoritariamente baseada em alimentos caracterizados pelo alto teor de gorduras saturadas, açúcares simples, com pouca ou nenhuma inclusão de vegetais (BUETTNER et al., 2007; GARDNER; RHODES, 2009). Desta forma, medidas para controlar a origem destas doenças crônicas por meio da alimentação vêm sendo cada vez mais o foco de investigações científicas.

A obesidade está associada a um processo inflamatório de baixo grau, porém crônico, que por sua vez é o principal responsável pela instalação da resistência à insulina (RI) (POSEY et al., 2009). Danos na sinalização da insulina

são fundamentais para o desenvolvimento de diabetes mellitus tipo 2 (DM2), e estão fortemente associados com a síndrome metabólica (MOLLER; KAUFMAN, 2005), neurodegeneração, e perdas na cognição, sintomas que predispõe o surgimento da doença de Alzheimer (CORREIA et al., 2012; STEEN et al., 2005).

As frutas vermelhas ou “berries” possuem uma concentração expressiva de polifenóis e pigmentos responsáveis por ação anti-obesogênica, antidiabetogênica e melhora dos quadros de RI e inflamação (DRAGANO et al., 2013). A capacidade dos compostos fenólicos em atravessarem a barreira hematoencefálica (BHE) propicia sua atividade neuroprotetora, permitindo ação direta no tecido nervoso (MILBURY; KALT 2010; SHUKITT-HALE, 2012). A atividade protetora destes compostos é relacionada, principalmente à sua capacidade de combater radicais livres e o estresse oxidativo, que está aumentado nos processos patológicos (KIM et al., 2010; GUO et al., 2012).

O açaí (*Euterpe sp*.) é um fruto tipicamente brasileiro, mais propriamente da Amazônia, e vem sendo consumido cada vez mais por todas as outras regiões do país e no mundo. Muita atenção lhe é dada devido à sua capacidade antioxidante e possível papel como alimento ou ingrediente funcional (SCHAUSS et al., 2006; COÏSSON et al., 2005). Esta fruta fornece vários compostos antioxidantes tais como polifenóis, carotenoides, e ácido ascórbico (SCHAUSS et al., 2006a). Estudos *in vitro* demonstraram que por possuir expressiva capacidade antioxidante, o açaí possui atividade hipocolesterolemiante *in vivo*, além de inibir a peroxidação lípidica em seres humanos (SCHAUSS et al., 2006; PACHECO-PALENCIA et al., 2008; SOUZA et al., 2012; SCHAUSS et al., 2009).

No presente trabalho, realizou-se o estudo da associação da ingestão de uma dieta hiperlipídica com a polpa de açaí, rica em fibras dietéticas, ácidos graxos insaturados e polifenóis, que são compostos associados com melhoras de parâmetros de saúde como exposto acima, com o objetivo de descobrir o potencial desta fruta em prevenir, além de marcadores metabólicos responsáveis por obesidade e RI, os fatores de risco para doença de Alzheimer relacionados à obesidade.

OBJETIVOS

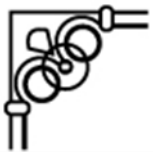
O objetivo geral deste trabalho foi investigar em camundongos o efeito da ingestão de polpa liofilizada de açaí (PLA) sobre o controle e prevenção de eventos fisiopatológicos relacionados à obesidade, com foco no comprometimento cognitivo.

Como objetivos específicos, este trabalho apresentou os seguintes fins:

- Avaliar características físico-químicas da polpa liofilizada de açaí, bem como seu valor nutricional e compostos bioativos;
- Comparar efeitos do consumo de dietas normolipídicas e hiperlipídicas sem e com adição de 2% de PLA;
- Avaliar o efeito funcional da ingestão da PLA sobre a resistência à insulina, obesidade e cognição e relacionar estes parâmetros;
- Analisar a eficácia do consumo de polpa liofilizada de açaí em atenuar os efeitos nocivos de dieta rica em gordura sobre o estresse oxidativo e glicemia dos animais;
- Determinar efeito da suplementação de PLA na prevenção de déficits cognitivos induzidos pela ingestão de dieta hiperlipídica nas proteínas de uma das vias de ativação da fosforilação da tau no hipocampo dos animais.

CAPÍTULO 1

REVISÃO DE LITERATURA



CAPÍTULO 1

REVISÃO DE LITERATURA

1 OBESIDADE: ETIOLOGIA E CONSEQUÊNCIAS PARA A SAÚDE

1.1 Fatores epidemiológicos da obesidade

A obesidade vem sendo considerada por muitos uma epidemia do século XXI e pode ser definida como um peso corporal desproporcional para a altura com acumulação excessiva de tecido adiposo, geralmente acompanhada de inflamação sistêmica leve e crônica (SELLAYAH; CAGAMPANG; COX, 2014). Apesar de ser uma doença multifatorial, que envolve fatores genéticos e endócrinos, também está associada a fatores comportamentais como a ingestão excessiva de dietas ricas em gorduras saturadas e açúcares e o baixo nível de atividade física (MENDONÇA, 2004). Estudos mostram que cerca de 12% da ingestão diária de energia dos adultos americanos vem de gorduras saturadas e 13% vem dos açúcares adicionados (MICHA et al., 2014). Este número é significantemente mais alto do que o recomendado (de 5-10%) pelo Departamento de Agricultura dos EUA (USA, 2015). Não surpreendentemente, os EUA são hoje o país com maior prevalência de sobrepeso e obesidade no mundo (OECD, 2016).

Estes dados são preocupantes, sendo a obesidade uma doença crônica de difícil tratamento e, apesar de décadas de esforços para retardar o progresso da epidemia, atualmente 39% da população mundial está obesa ou com excesso de peso (WHO, 2015). No Brasil, pesquisa realizada em 2016 pela VIGITEL (Vigilância de Fatores de Risco e Proteção para Doenças Cardiovasculares por Inquérito Telefônico) revela que 53,8% dos brasileiros estão acima do peso e quase 19% já tem um quadro de obesidade estabelecido, de acordo com o IMC (BRASIL, 2017). A situação é ainda mais alarmante quando se avalia a evolução do sobrepeso e obesidade ao longo dos anos (2006 – sobrepeso: 43% e obesidade: 11%), sendo observado aumento dessas no decorrer do tempo (BRASIL, 2017).

A epidemia de obesidade tem implicações psicossociais e econômicas relevantes, representando uma importante fonte de custos de Saúde Pública. Em

2002, os Estados Unidos tiveram um gasto de em média 92,6 bilhões de dólares com a obesidade e o sobrepeso, o equivalente a 9% do total das despesas com saúde no país (NGUYEN; EL-SERAG, 2010). Além de ser por si só, um problema de saúde pública mundial, as consequencias da obesidade para a saúde são inúmeras. A obesidade é um fator de risco para diversas doenças como as doenças cardiovasculares, diversos tipos de câncer, síndrome metabólica, DM2 e, mais recentemente, também tem sido associada a insuficiências cognitivas e demência (MILLER; SPENCER, 2014). Desta maneira, diversos estudos vem sendo elaborados nesta área com a intenção de impulsionar o desenvolvimento de novas abordagens para a compreensão, prevenção e combate aos fatores de risco para estas doenças.

1.2 Tecido adiposo, inflamação e resistência à insulina

O tecido adiposo é um órgão endócrino metabolicamente ativo, que se comunica com o sistema nervoso central (SNC) e com tecidos periféricos por meio da secreção de adipocinas (p. ex. leptina, adiponectina), e citocinas (p. ex. TNFα, IL-1β, IL-6 e quimiocinas) que regulam vários processos metabólicos, bem como a homeostase energética, sensibilização à insulina e resposta imuno-inflamatória (OTTAVIANI; MALAGOLI; FRANCESCHI, 2011). O consumo calórico excessivo ocasiona um aumento do acúmulo de triglicerídeos nos adipócitos (hipertrofia) e, em algumas situações, em número (hiperplasia) dos adipócitos, gerando uma disfunção nestes. Os adipócitos hipertrofiados secretam agentes pró-inflamatórios promovendo inflamação sistêmica de baixo grau (KENNEDY et al., 2008).

O aumento de tecido adiposo pode aumentar também a infiltração de citocinas pró-inflamatórias (p. ex. TNFα, IL-6) após estabelecido quadro inflamatório no tecido devido à infiltração de macrófagos, característica da obesidade (OTTAVIANI; MALAGOLI; FRANCESCHI, 2011). Várias serina/treonina quinases são ativadas por citocinas pró-inflamatórias que, juntamente com os altos níveis de ácidos graxos livres circulantes, contribuem para a inibição da sinalização da insulina ao propagar sinais de fosforilação do substrato 1 do receptor da insulina (IRS-1) em serina (MILANSKI et al., 2012). Sendo assim, o aumento de células adiposas e a infiltração por macrófagos no tecido adiposo são os principais

contribuintes para a inflamação e a resistência à insulina (RI). As citocinas produzidas podem atingir a circulação e prejudicar a propagação do sinal da insulina em outros órgãos como fígado e músculos (HOTAMISLIGIL, 2006).

A RI é considerada um estado metabólico no qual há diminuição da capacidade da insulina endógena ou exógena em estimular a utilização celular de glicose e manter normais as suas respostas metabólicas (REAVEN, 2005). Como mecanismo compensatório, as células β-pancreáticas passam a produzir mais insulina a fim de manter a homeostase glicêmica, ocorrendo a falência progressiva destas células. A RI, causada pela obesidade, combinada com secreção insuficiente de insulina pelas células β-pancreáticas são a principal causa de DM2 (JOHNSON; OLEFSKY, 2013; KAHN; HULL; UTZSCHNEIDER, 2006; KUSMINSKI et al., 2009).

1.3 Produção de adipocinas na obesidade

A leptina regula o comportamento alimentar quando sinaliza estímulos anorexigênicos (supressores da fome) no hipotálamo e possui ainda características pró-inflamatórias (FRIEDMAN; HALAAS, 1998; TILG; MOSCHEN, 2006). Na obesidade a produção das adipocinas encontra-se desequilibrada devido a inflamação e hipertrofia do tecido (TILG; MOSCHEN, 2006). Os níveis de leptina no sangue correlacionam-se positivamente com a massa adiposa, sendo que, nos indivíduos obesos, ocorre uma alta produção de leptina, condição conhecida por hiperleptinemia, que induz a falhas na sua sinalização, indicando a ocorrência de resistência à leptina, e consequentemente menor atuação de neurotransmissores anorexigênicos, agravando o quadro de obesidade (FRIEDMAN; HALAAS, 1998; MYERS; COWLEY; MÜNZBERG, 2008).

A adiponectina é outra adipocina secretada pelo tecido adiposo. Possui características anti-inflamatórias, sendo capaz de interagir, em condições normais, com células imunes como macrófagos, onde suprime a produção e secreção de TNFα e IL-6 e monócitos, aumentando sua produção de citocinas anti-inflamatórias (GARAULET et al., 2007). Ela melhora a sensibilidade à insulina, oxidação da gordura muscular e dissipação energética (JUGE-AUBRY; HENRICHOT; MEIER, 2005). A ação sobre a sensibilidade à insulina da adiponectina pode envolver a ativação da proteína quinase dependente de AMP (AMPK), conhecida por regular a

produção de glicose no fígado pela diminuição da expressão de enzimas da neoglicogênese (KERSHAW; FLIER, 2004). Os níveis de adiponectina no plasma e no tecido adiposo diminuem em indivíduos obesos, onde é inibida tanto pelos fatores pró-inflamatórios, como TNFα e IL-6, bem como por hipóxia e pelo estresse oxidativo (HOSOGAI et al., 2007).

2 O PAPEL DA OBESIDADE NO DESENVOLVIMENTO DE DOENÇAS NEURODEGENERATIVAS

2.1 Doença de Alzheimer e obesidade

A doença de Alzheimer (DA) é a sexta principal causa de morte nos Estados Unidos, com cerca de 5,5 milhões de pessoas com a doença (MURPHY et al., 2016). É o subtipo mais comum de demência, com uma prevalência global de 10-30% na população acima dos 65 anos de idade, representando de 60% a 90% de todas as demências. Estima-se que havia 46,8 milhões de pessoas em todo o mundo vivendo com demência em 2015 e que este número alcançará 131,5 milhões em 2050 (PRINCE et al., 2016). Com o envelhecimento da população e o consequente aumento na incidência da DA, esta doença tornou-se um desafio para o sistema de saúde, com um custo estimado anual de US$ 259 bilhões (REITZ; MAYEUX, 2014).

A DA é neurodegenerativa e progressiva, diagnosticada principalmente por suas características clínicas, incluindo comprometimento progressivo da função cognitiva, orientação e atividade motora, distúrbios da fala, dificuldade em reconhecer ou identificar objetos e pessoas (MASTERS et al., 2015). As características histopatológicas da DA incluem as chamadas placas senis e os emaranhados neurofibrilares (NFT) formados, respectivamente, por deposição do peptídeo amiloide-β agregado (Aβ), um produto de clivagem da proteína precursora da amiloide (APP), e da agregação da proteína estabilizante de microtúbulo tau (tau) hiperfosforilada. Juntamente com processos associados, como inflamação e estresse oxidativo, estas características patológicas contribuem para a perda da integridade sináptica e neurodegeneração progressiva (BLENNOW; DE LEON; ZETTERBERG, 2006).

A alta ingestão de gordura e a obesidade têm sido associadas com a patogênese da neurodegeneração e DA (LUCHSINGER et al., 2002). Estudos sugerem que a obesidade e o aumento do consumo de dietas com alto teor de gordura aumentam o risco de desenvolvimento de demência (ELIAS et al., 2003; KALMIJN et al., 1997; SOLFRIZZI; PANZA; CAPURSO, 2003). Em 1990, Greenwood e Winocur publicaram um dos primeiros estudos que revelou os efeitos do consumo de dieta rica em ácidos graxos saturados (SFA) na aprendizagem e memória em ratos (GREENWOOD; WINOCUR, 1990).

A RI e o DM2 estão intimamente relacionados ao surgimento de doenças neurodegenerativas e ao desenvolvimento de demência, sendo este o principal motivo pelo qual o excesso de peso e a obesidade são considerados fatores de risco para o desenvolvimento de DA (CHENG et al., 2012; HENI et al., 2015). Além disso, níveis elevados de citocinas pró-inflamatórias aumentam a inflamação, o que, por sua vez, causa déficit cognitivo (UCHOA; MOSER; PIKE, 2016).

2.2 Consequências da obesidade nos marcadores cognitivos

Estudos com modelos de obesidade induzida por dieta em roedores demonstram comprometimento no desempenho da memória e na aprendizagem (BOITARD et al., 2014; KOTHARI et al., 2017; SAH et al., 2017). Esses efeitos são atribuídos principalmente à inflamação e alteração da ação da insulina no cérebro (DE FELICE; FERREIRA, 2014; PISTELL et al., 2010). A inflamação cerebral é caracterizada por níveis elevados de citocinas inflamatórias segregadas por células locais (células da micróglia) (HARRY; KRAFT, 2008; MANDREKAR; LANDRETH, 2010). Além disso, as citocinas pró-inflamatórias secretadas em alta quantidade pelo tecido adiposo na obesidade podem ter acesso ao cérebro através da BHE e complementar a resposta inflamatória local, resultando em plasticidade sináptica reduzida e neurogênese prejudicada (KILIAAN; ARNOLDUSSEN; GUSTAFSON, 2014; ZHENG; ZHOU; WANG, 2016).

As células do cérebro não dependem totalmente de insulina para o fornecimento de glicose, na medida em que possuem outros meios para sua obtenção. Em contraste com os tecidos periféricos, o cérebro é considerado um órgão insensível à insulina porque o GLUT-4 (transportador de glicose dependente

de insulina) está presente em baixo nível em algumas regiões do cérebro em comparação com as outras isoformas, GLUT-1 e GLUT-3, e não parece ser significativamente regulado pela insulina (BLÁZQUEZ et al., 2014).

Além disso, a insulina é produzida quase exclusivamente pelas células β-pancreáticas e, sendo uma proteína, supunha-se que não poderia atravessar a BHE, o que sustentava a ideia de que o SNC era um tecido insensível à insulina (BANKS; OWEN; ERICKSON, 2012). No entanto, receptores da insulina (IR) e seus substratos podem ser encontrados em diversas regiões do cérebro. A insulina é transportada pela BHE por um sistema de transporte saturável. Um número crescente de funções não metabólicas para a insulina no SNC estão sendo descobertas em regiões relacionadas com a memória e aprendizagem: hipocampo, amígdala e córtex frontal (BANKS; OWEN; ERICKSON, 2012; ZHAO; ALKON, 2001). Nestas regiões, o estímulo da insulina é importante para funções como a sobrevida de neurônios, expressão da tau, metabolismo energético, função mitocondrial, atividades sinápticas e processo cognitivo (DE LA MONTE, 2008; MESSIER; TEUTENBERG, 2005).

Em uma condição normal, com a sinalização da insulina, o IRS é fosforilado em tirosina e transmite sinais intracelulares de ativação da via da fosfatidil-inositol 3-kinase (PI3K)- proteína kinase B (PKB/AKT). A PI3K estimula o transporte de glicose e inibe a apoptose ativando PKB/AKT, que inibe a glicogênio sintase quinase-3β (GSK-3β) em Serina 9, uma importante quinase de fosforilação da tau (DUDEK; DATTA, 1997; LEE; KIM, 2007). Na RI, o bloqueio da propagação do sinal da insulina inativa a quinase AKT, que se torna incapaz de fosforilar GSK3-β e bloquear a sua ação de fosforilar a tau e impedir sua agregação e consequente formação de NFT (CORREIA et al., 2012; JOLIVALT et al., 2008; SCHUBERT et al., 2003).

Na obesidade, os níveis elevados de citocinas pró-inflamatórias podem bloquear a sinalização de insulina intracelular, afetando o IRS-1 ao propagar sinais de fosforilação em serina (DE FELICE; FERREIRA, 2014). A expressão e/ou ação diminuída de IRS-1 no hipocampo foram identificadas em indivíduos com DA (TALBOT et al., 2012). Além disso, indivíduos portadores de DA tem a taxa de líquido cefalorraquidiano / insulina periférica reduzida em comparação à indivíduos saudáveis, indicando redução da ação da insulina no SNC (FREIHERR et al., 2013;

KLEINRIDDERS et al., 2014). A alteração da ação da insulina no cérebro parece estar envolvida tanto com a acumulação de Aβ quanto com a hiperfosforilação da tau e consequente formação dos NFT (Figura 1). Destacaremos aqui a ocorrência dos NFT, uma vez que se correlaciona com o grau de comprometimento cognitivo da DA (EL KHOURY et al., 2014; NELSON ET AL., 2013).

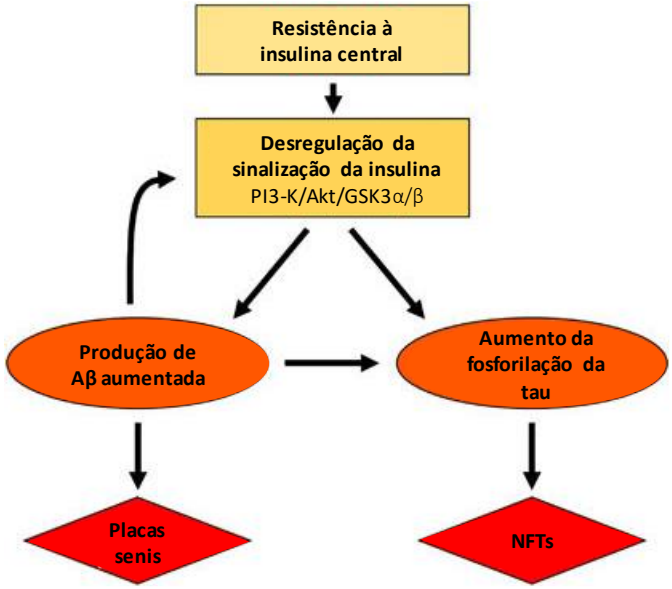


Figura 1- Resistência à insulina periférica induz a resistência à insulina central no cérebro. A sinalização de insulina prejudicada resultante, que afeta principalmente a via PI3K/AKT, então aumenta os níveis de processamento de APP/Aβ e fosforilação de tau. Finalmente, o aumento da Aβ interrompe ainda mais a sinalização de insulina para exacerbar a patologia do AD e o declínio cognitivo. Adaptado de (KIM; FELDMAN, 2015).

A proteína tau é responsável por estabilizar os microtúbulos, que são estruturas proteicas que compõem o citoesqueleto de neurônios e axônios predominantemente (HIMMLER et al., 1989). A tau é regulada por muitas quinases e fosfatases diferentes que modificam seu estado de fosforilação e capacidade de ligação a microtúbulos (AUGUSTINACK et al., 2002; CAVALLINI et al., 2013; MORISHIMA-KAWASHIMA et al., 1995). Quando a tau se torna hiperfosforilada em vários sítios, não consegue mais se ligar e estabilizar os microtúbulos, desestabilizando-o e assim se agrupando no citosol. Ao se agrupar, tende a formar os filamentos helicoidais pareados (PHFs) e em sequência compor os NFTs (MOKHTAR et al., 2013). Esses emaranhados são observados principalmente no hipocampo, no córtex entorrinal e na amígdala, principais regiões afetadas na DA

(LEWIS et al., 2000). O axônio é degenerado com a deterioração do citoesqueleto, perdendo a capacidade de manter as conexões e sinapses dos neurônios (MATTSON, 1995).

Os NFTs são uma característica clássica da tauopatia observada não somente na DA, mas em outras doenças neurodegenerativas como demência frontotemporal (FTD), doença de Parkinson, entre outras (KOSIK; JOACHIM; SELKOE, 1986; WILLIAMS, 2006; WOOD et al., 1986). Embora não esteja claro se as NFTs induzem diretamente a neurodegeneração, sua presença está associada à morte neuronal (KRIL et al., 2002). Como a sinalização de insulina pode modular a fosforilação da proteína tau, uma falha da sua ação no cérebro poderia levar à diminuição da função neuronal e sinaptogênese (KLEINRIDDERS et al., 2014). Assim, o desenvolvimento de RI especifica do cérebro fundamenta a ideia de que a doença de Alzheimer seja um terceiro tipo de diabetes (DE LA MONTE, 2008).

Em um mecanismo adicional, falhas na sinalização da leptina também podem levar à hiperfosforilação da tau e à neurodegeneração (LEE, 2011). Apesar dos altos níveis periféricos circulantes de leptina, os níveis de leptina do líquido cefalorraquidiano também parecem estar mais baixos na obesidade, sugerindo o transporte prejudicado da leptina através da BHE e um mecanismo local de resistência à leptina (FARR; TSOUKAS; MANTZOROS, 2015). Os receptores de leptina são encontrados não apenas no hipotálamo, que é o principal sítio de ação da leptina na regulação do peso corporal, mas também são expressos no córtex e no hipocampo, duas áreas principais afetadas em DA (HÅKANSSON; MEISTER, 1998). Nas células neuronais, demonstrou-se que a leptina reduz significativamente a fosforilação de tau em várias regiões vulneráveis do cérebro e melhora a patologia do cérebro relacionada à Aβ e à tau (GRECO et al., 2008). Em modelos de ratos com DA, a leptina parece melhorar a neurogênese do hipocampo pela proliferação de precursores neuronais e atenuar a neurodegeneração induzida por Aβ (GRECO et al., 2010). Por fim, a leptina parece ter efeitos neurotróficos e neuroprotetores agudos no modelo de camundongo transgênico APP/PS1 (PÉREZ-GONZÁLEZ et al., 2011).

2.3 Utilização do teste de reconhecimento de objeto novo como medida de memória em modelos animais

Os modelos animais de memória são o objeto de muitas publicações científicas, pelo menos desde o século XX (GALLAGHER, 1997; MORRIS et al., 1982). Nos seres humanos, a memória é acessada através de linguagem falada ou escrita, enquanto que nos animais, as funções cognitivas devem ser acessadas através de diferentes tipos de comportamentos em muitos modelos específicos de memória e aprendizado (ANTUNES; BIALA, 2012).

O teste de reconhecimento de objeto novo (NOR) é usado para avaliar a cognição, particularmente a memória de reconhecimento, em modelos de distúrbios do SNC em roedores. O teste explora o instinto natural dos roedores por ter interesse pela novidade e tornou-se um modelo amplamente utilizado para a investigação de alterações de memória (BAXTER, 2010). A medida do reconhecimento de objeto é influenciada pela diferença entre o tempo gasto com um novo objeto e o tempo gasto com um objeto familiar (ENNACEUR; DELACOUR, 1988). O teste é composto por três fases: habituação, familiarização e fase de teste. Na fase de habituação, cada animal explora livremente uma arena na ausência de objetos e em seguida retorna para sua gaiola. Durante a fase de familiarização, o animal é colocado na arena contendo dois objetos idênticos e deixado explorá-los durante alguns minutos. Para evitar a coerção na exploração dos objetos, os roedores são colocados de costas para o objeto. Após um intervalo de retenção, durante a fase de teste, o animal é colocado novamente na arena com dois objetos, mas agora um é idêntico ao anterior e o outro é novo (Figura 2) (ENNACEUR, 2010; ENNACEUR; DELACOUR, 1988; GASKIN et al., 2010; HAMMOND; TULL; STACKMAN, 2004; TAGLIALATELA et al., 2009). Tanto na fase de familiarização como na fase de teste, os objetos estão localizados em cantos opostos e simétricos da arena e a localização do objeto novo versus familiar é contrabalançada (HAMMOND; TULL; STACKMAN, 2004). Os roedores saudáveis passam mais tempo explorando o novo objeto durante os primeiros minutos da fase de teste em detrimento do antigo, sendo que se o contrário acontece, há algum indicativo de comprometimento cognitivo (ENNACEUR, 2010).

Figura 2 – Teste de reconhecimento de objetos (NOR): os animais são deixados em uma caixa para explorar o ambiente livremente sem objetos na fase de habituação; na fase de familiarização dois objetos idênticos são colocados na arena para que os animais explorem livremente. Na terceira fase (fase de teste) um dos objetos é trocar por um objeto novo e a preferência por um dos objetos em relação ao tempo total de exploração é medida.

O hipocampo é fundamental para a memória de reconhecimento de objetos e, se houver lesões nesta estrutura, ocorrerá comprometimento moderado da memória anterógrada – a capacidade de armazenar novas informações a partir de um determinado momento (BROADBENT et al., 2010). Embora o hipocampo não desempenhe um papel direto na discriminação das diferentes características de cada objeto, é fundamental como um “detector de novidades” devido ao seu papel na comparação de informações armazenadas anteriormente com os novos aspectos recebidos de uma situação particular (CLARKE et al., 2010).

3 ATUAÇÃO DOS COMPOSTOS BIOATIVOS NA SAÚDE

3.1 Frutas vermelhas e seus compostos bioativos

As plantas produzem grande quantidade de compostos fenólicos. De acordo com as suas estruturas químicas, estes polifenóis são divididos em várias famílias, uma das quais é a família dos flavonoides (MANACH et al., 2004). As plantas comestíveis, bem como alimentos e bebidas derivadas delas, proporcionam à dieta humana generosas quantidades de flavonoides (até 1g/dia), por exemplo (CROZIER; JAGANATH; CLIFFORD, 2007). Os flavonoides têm uma estrutura química de base constituída por dois anéis de benzeno ligados através de um anel de pirano heterocíclico. Os padrões substituintes do grupo hidroxila fornecem os centros de reação. Os flavonoides podem ser divididos em várias subfamílias de acordo com o grau de oxidação do heterociclo de oxigênio e os padrões de

substituição, por exemplo, flavonas, flavonóis, isoflavonas, antocianinas, flavonoides, e flavanonas (CROZIER; JAGANATH; CLIFFORD, 2007; JAGANATH; CROZIER, 2011).

As antocianinas são responsáveis pela pigmentação vermelha, azul e violeta de flores, frutos, folhas, sementes e raízes. Pertencem à grande classe dos compostos fenólicos, sendo classificadas secundariamente como flavonoides, devido à estrutura característica de sua cadeia carbônica, na qual dois anéis aromáticos estão separados por um anel heterocíclico (KONG et al., 2003). São compostos de rápida absorção e metabolização, associados não só a ações antioxidantes, como a ações anti-inflamatórias, anti-obesogênicas e sensibilizadora à insulina in vivo (DRAGANO et al., 2013).

3.2 Mecanismos de ação na resistência à insulina e neurodegeneração

Mecanismos de ação contra a RI: a cianidina-3-glicosídeo (C3G) pode interferir na produção hepática de glicose, que é um fator importante no diabetes. Reduz a expressão de TNF-α e outras citocinas pró-inflamatórias no fígado e tecido adiposo, além de inibir a ativação de JNK. Ao aumentar a fosforilação de AKT, a C3G estimula a diminuição na produção hepática de glicose pela diminuição de FoxO1 no núcleo dos adipócitos e hepatócitos (DRAGANO et al., 2013; GUO et al., 2012). Sob estímulo da C3G, a AKT fosforilada pode ainda ativar a proteína AS160. Esta ativa a translocação do transportador de glicose GLUT4 até a membrana para captação da glicose em células dos tecidos adiposo e muscular (LETO; SALTIEL, 2012). Como um mecanismo paralelo, antocianinas também podem doar elétrons e neutralizar os radicais livres, impedindo a ativação de JNK pelo estresse oxidativo (PRIOR, 2003).

Em ratos alimentados com uma dieta de alto teor de frutose, a suplementação com polifenóis “não flavonoides” também mostrou aumento na expressão de RNAm e nos níveis de proteínas de sinalização de insulina (por exemplo: receptores de insulina, IRS-1 e 2, PI3K, AKT e transportadores de glicose GLUT 1 e 4) (QIN et al., 2010).

Mecanismos de ação nos núcleos centrais de cognição: as frutas vermelhas, ricas em flavonoides, especialmente antocianinas, tem sido associadas à

prevenção e desaceleração de doenças neurodegenerativas. Atividades anti-inflamatórias neurais e sistêmicas de flavonoides têm sido relatadas e podem ser associadas com a prevenção de muitas doenças crônicas (DEVORE et al., 2012).

Cada vez mais tem crescido as evidências de que o consumo de alimentos ricos em flavonoides pode influenciar positivamente a função cognitiva, bem como inibir a progressão da DA e/ou reverter os déficits cognitivos em modelos animais, especialmente roedores. Estes dados sugerem que estes compostos bioativos tenham potencial terapêutico na prevenção e/ou tratamento da demência (BATISTA et al., 2017; CAREY et al., 2017; CAREY; GOMES; SHUKITT-HALE, 2014; WILLIAMS; SPENCER, 2012). O maior consumo de “berries” e antocianinas, bem como flavonoides totais por mulheres mais velhas, foi associado à progressão mais lenta de declínio cognitivo (DEVORE et al., 2012).

Trabalhos com modelos experimentais sugerem que frutas vermelhas ricas em antocianinas atenuam a peroxidação lipídica e elevaram o status antioxidante no cérebro de animais obesos, mesmo com diminuição na massa cerebral (BATISTA et al., 2014). Adicionalmente, após a suplementação de mirtilo selvagem, rico em flavonoides e antocianinas, houve significante melhora na capacidade de memória e aprendizado de roedores durante teste cognitivo de esquiva-passiva (PAPANDREOU et al., 2009). Para corroborar estes resultados, os autores observaram diminuição na atividade de acetilcolinesterase (uma enzima que quebra a acetilcolina, neurotransmissor encontrado no cérebro), aumento nos níveis de glutationa e redução da peroxidação lipídica no cérebro dos camundongos (PAPANDREOU et al., 2009). O mirtilo também induziu diminuição nos níveis de substâncias pró-oxidantes, de apoptose e neurotoxicidade no tecido cerebral causado por adição de galactose em dieta de ratos experimentais (ÇOBAN et al., 2014).

Em relação ao mecanismo molecular de atuação destes compostos, sabe-se que os flavonoides podem se ligar a receptores ou mediadores de vias de sinalização da PI3K, exercendo neuroproteção por suprimir a ativação transcricional da FoxO1 para genes apoptóticos (Fas ligante), além de inibir a hiperfosforilação da tau mediada por GSK3β, mantendo a função dos axônios (WILLIAMS; SPENCER, 2012). As vias de ativação da inflamação, da leptina e AMPK nos centros nervosos, também são outro ponto de ação dos flavonoides nos centros de controle da

cognição. Estes podem sensibilizar a ação da insulina, e aumentar a taxa de fosforilação da GSK3β e impedir a fosforilação da tau (GRECO et al., 2008, 2009a, 2009b; JEON et al., 2012). Outros mecanismos de atuação dos flavonoides nos núcleos centrais são relatados no que diz respeito à inibição da produção da proteína Aβ, impedindo a formação das placas senis (DRAGICEVIC et al., 2011).

4 AÇAÍ

O açaí (*Euterpe* sp.) é uma fruta típica da Amazônia, com grande ocorrência e importância econômica no estado brasileiro do Pará. O açaizeiro é uma palmeira delgada e de múltiplas dimensões, amplamente distribuída nas planícies de inundação do estuário amazônico (MUÑIZ-MIRET et al., 1996). Seu fruto é altamente perecível, portanto é predominantemente consumido e comercializado como polpas congeladas e outros produtos industriais, que passam por várias etapas de processamento, como pasteurização, congelamento e diluição (PACHECO-PALENCIA; HAWKEN; TALCOTT, 2007). A polpa comestível de açaí é comumente macerada com água para produzir uma bebida espessa e roxa de textura cremosa, aparência oleosa e sabor característico, o “vinho” - nome dado à polpa do açaí diluída em pouca água - tradicional na cultura dos povos da região (MUÑIZ-MIRET et al., 1996).

Devido à sua natureza altamente perecível, o consumo e a comercialização do açaí eram restritos a apenas um nível regional no Brasil. No entanto, o aumento do interesse internacional e a expansão da sua distribuição tornaram a polpa de açaí e vários produtos de varejo feitos de polpa de açaí amplamente disponíveis para o público em geral. Além de ser uma fruta altamente energética, o açaí é reconhecido por suas propriedades funcionais para uso em produtos nutricionais e alimentos, devido à sua alta atividade antioxidante, relacionada ao alto teor antocianico e fenólico (COÏSSON et al., 2005; SCHAUSS et al., 2006b).

Duas espécies de açaí são cultivadas e apresentam grande valor econômico para os moradores da região Amazônica: *Euterpe precatoria* e *Euterpe oleracea*. A principal diferença entre elas é seu padrão de crescimento. A primeira é uma palmeira de um único caule (estipe, como é chamado), e a *E. oleracea* é uma

palmeira multicaule, com cerca de treze plantas (Figura 3). A *E. precatoria* atinge, em média, 15 a 35 m de altura e 10 a 20 cm de diâmetro, enquanto a *E. oleracea* alcança 15 a 20 m de altura e 12 a 18 cm de diâmetro. O cacho pode ter peso entre 3 e 8 kg, sendo que 70% destes corresponde ao peso dos frutos. Os frutos são drupas de forma globosa, roxo escuro, com diâmetro de aproximadamente 1,7 cm e peso entre 2 e 3 g, sendo cerca de 7% polpa (Figura 4). Seu fruto maduro tem coloração de púrpura a quase preta e podem ser colhidos o ano todo, mas apresentam melhor qualidade organoléptica de agosto a dezembro (LORENZI; DE SOUZA, 1996; ROGEZ, 2000).

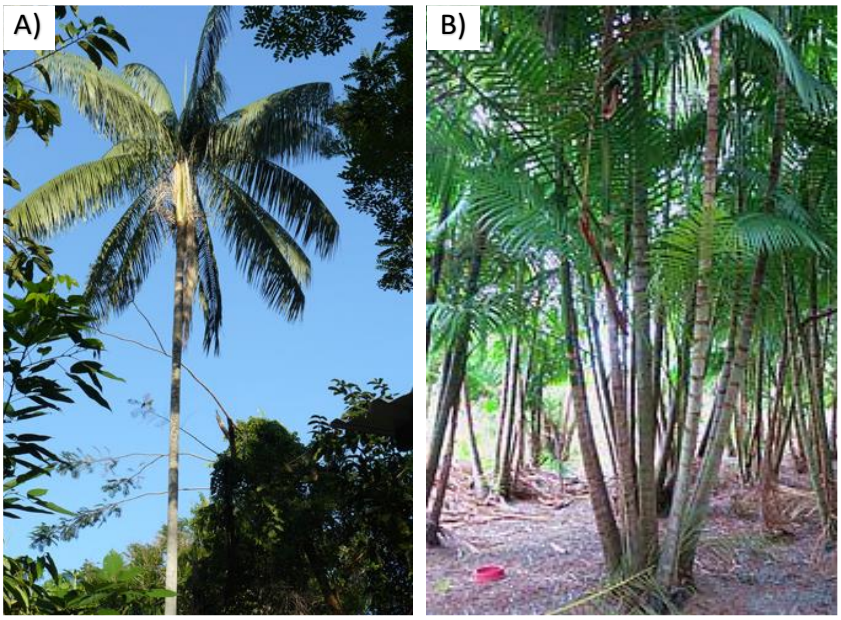


Figura 3 – Hábito de crescimento monocaule da palmeira de *E. Precatoria* (A) e multicaule da palmeira de *E. Oleracea (B)*. Fotos: Google Imagens; reprodução.

Muita atenção é dada ao açaí devido ao seu potencial antioxidante e seu possível papel como alimento funcional. O açaí apresenta entre seus constituintes nutricionais alto teor de energia, devido principalmente à presença de lipídios, tornando-o um importante alimento de suprimento energético. Ao contrário da maioria das frutas e bagas, ou "*berries*", ele é fonte de ácidos graxos monoinsaturados, principalmente o oleico, mas encontra-se também quantidades consideráveis dos ácidos graxos essenciais poli-insaturados, como o linoleico e linolênico (YUYAMA et al., 2011).

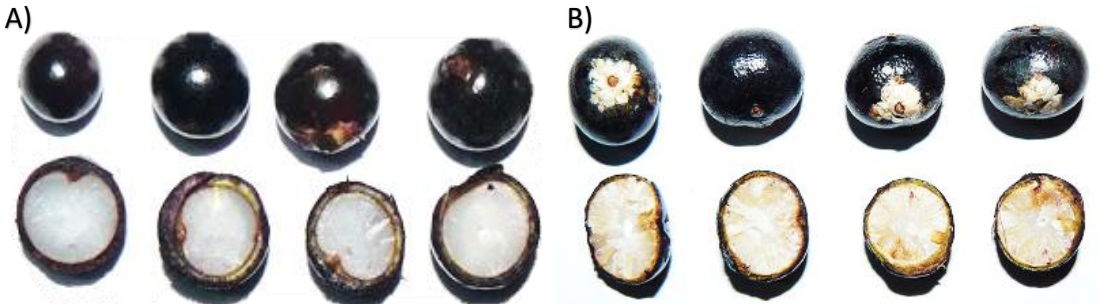


Figura 4 – Frutos inteiros e descerrados de *E. Precatoria* (A) e *E. Oleracea (B)*. Fotos: Frutas Nativas da Amazônia. Fonte: http://frutasnativasdaamazonia.blogspot.com.br.

Além de seu valor nutricional como fonte de energia, é expressivo o seu teor de fibra alimentar e de antocianinas. Entre as antocianinas, cianidina-3-O-rutinosídeo e cianidina-3-O-glicosídeo são relatadas como as principais constituintes deste fruto (GALLORI et al., 2004; PACHECO-PALENCIA; HAWKEN; TALCOTT, 2007; SCHAUSS et al., 2006a). Estudos realizados têm associado o fruto à, além da ação antioxidante, atividade pró-apoptótica em células cancerígenas, hipocolesterolemiante e anti-inflamatória em modelos *in vitro* e *in vivo* (CHOI et al., 2017; DE OLIVEIRA et al., 2015; DIAS et al., 2015; SCHAUSS et al., 2006b; SILVA et al., 2014) . Além disso, o açaí foi relacionado com a proteção de células neuronais *in vitro* contra o estresse oxidativo induzido e ataques inflamatórios de regiões envolvidas tanto na função cerebral geral, quanto na memória e cognição e na reversão dos efeitos prejudiciais do envelhecimento no comportamento motor e cognitivo em animais idosos (CAREY et al., 2017; CAREY; GOMES; SHUKITT-HALE, 2014). Não surpreendentemente, os povos indígenas das áreas nativas frequentemente utilizam este fruto na medicina popular (SCHRECKINGER et al., 2010). A Tabela 1 ilustra os compostos e quantidades encontradas no fruto de açaí já relatados na literatura.

Tabela 1- Composição química, físico-química e atividade antioxidante do açaí.

Composto/Atividade	**Parte da fruta**	**Valor**	**Método**	**Ref.**
Umidade (g%)	Polpa* (b. u.)	4,92	AOAC	1
Proteínas (g%)	Polpa* (b. u.)	8,13	IAL	1

Lipídios (g%)	Polpa* (b. u.)	40,75	AOAC	1
Cinzas (g%)	Polpa* (b. u.)	3,68	AOAC	1
Fibras (g%)	Polpa (b. u.)	5,5	Enzimático-gravimétrico	3
Carboidratos totais (g%)	Polpa* (b. u.)	42,53	Diferença	1
Energia (kcal%)	Polpa (b. u.)	489,39	Cálculo de conversão	1
Polifenóis (mg GAE/g)	Polpa (b. u.)	1109.5	Folin-ciocalteu	2
Flavonoides (mg /g)	Polpa (b. s.)	55,9	HPLC-MS	4
Antocianinas (mg%)	Polpa (b. u.)	303,3	HPLC-MS	5
AAO (µM trolox/g)	Polpa (b. u.)	117,2	DPPH	2
Sódio	Polpa* (b. u.)	28,5	Espectrofotometria de massa	1
Cálcio (mg%)	Polpa* (b. u.)	330	Espectrofotometria de massa	1
Ferro (mg%)	Polpa* (b. u.)	4,5	Espectrofotometria de massa	1

AAO= atividade antioxidante; b.u.= base úmida; b.s.= base seca; *liofilizada. [1]MENEZES et al. (2008); [2]BORGES et al. (2003); [3]YUYAMA et al. (2011); [4]RIBEIRO et al., (2010); [5]ROSSO et al., (2008).

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CAPÍTULO 2

Artigo Original: CONSUMO DE POLPA DE AÇAÍ MELHORA FUNÇÕES COGNITIVAS E SENSIBILIDADE PERIFÉRICA À INSULINA EM CAMUNDONGOS ALIMENTADOS COM DIETA HIPERLIPÍDICA

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Research article

Açai pulp intake improves cognitive functions and peripheral insulin sensitivity in high-fat diet-fed mice

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Abbreviations: AD: Alzheimer's disease; AKT: protein kinase B; AP: açai pulp; CAT: catalase; EAT: epidiymal adipose tissue; GSH: reduced glutathione; GSK3-β: Glycogen synthase kinase 3-β; IRS: insulin receptor substrate; MAT: mesenteric adipose tissue; NOR: novel object recognition; NFT: neurofibrillary tangles; RAT: retroperitoneal adipose tissue; SOD: superoxide dismutase; Tau: microtubule-associated protein tau;

ABSTRACT

Açai is a Brazilian berry rich in flavonoids, anthocyanins and other bioactive compounds involved in mechanisms related to several benefits to health, such as oleic acid. Many evidences indicate that obesity and insulin resistance constitute risk factors for development of neurodegenerative diseases. (Poly)phenol compounds are under study for its role in protecting effects against neural injuries and degeneration. In this regard, an Amazonian açai pulp (AP) was characterized due to its proximate composition, phenolic compounds, carotenoids, and antioxidant capacity. Then, we induced obesity and insulin resistance with a high-fat diet (H group) in mice and compared to a normal-fat standard diet (N group). We have supplemented the both diets with 2% AP and investigated proofs of peripheral insulin resistance, recognition memory, tau protein stability via AKT/GSK3-β signaling, and oxidative stress in hippocampus. The high-fat diet supplemented with AP (HA group) did not prevent weight gain, but improved peripheral insulin sensitivity and phosphorylation of AKT/GSK3-β in hippocampus, although no significant differences were observed in tau phosphorylation. The animals fed high-fat diets with AP showed better performance in novel object recognition test (NOR) in comparison to the H group. Catalase activity and reduced glutathione (GSH) values were improved in the HA mice. These results suggested that the supplementation of AP can attenuate the effects of a high-fat diet consumption in peripheral insulin resistance and improve cognitive behavior, but the mechanisms underlying this finding doesn’t seems to depend of tau phosphorylation.

Keywords: *Euterpe sp*.; super-fruit; neurodegeneration; anthocyanins; obesity.

1. Introduction

A long-term consumption of a high-energy, high-fat diets in middle or late life has been associated with an increased risk of cognitive deficits and neurological diseases such as dementia like Alzheimer's disease (AD) (Freeman et al., 2014; Kothari et al., 2017; Morris & Tangney, 2014; Spencer et al., 2017). Obesity and its resulting morbidities, such as diabetes mellitus type 2, midlife hypertension and sedentarism are primary preventable environmental risk factors associated to emergence of AD beyond old age and genetic factors (Norton et al., 2014). Consuming a high-fat diet may cause central nervous system dysfunction through multiple mechanisms, not completely elucidated so far.

The intake of high-fat diets, rich in saturated fatty acids, has been associated to behavioral deficits and increased phosphorylation of tau (microtubule-associated tau protein) in mouse (Kothari et al., 2017). Both insulin and leptin resistance due to obese conditions are pointed out as possible causes in the context (Kim & Feldman, 2015; Lee, 2011).

Glycogen synthase kinase-3β (GSK-3β) is the main tau kinase in the brain and in abnormal conditions, it is responsible for tau hyperphosphorylation and consequent formation of neurofibrillary tangles (NFT), an important marker of neuronal alterations in AD (Kaytor & Orr, 2002). Normally, this kinase is inactivated when phosphorylated at Serine 9 by other kinases, such as AKT (protein kinase B) (Kaytor & Orr, 2002; Schubert et al., 2004). Leptin appears to play a role in reduction of tau phosphorylation mediated through AMPK, AKT and p38 pathways (Greco et al, 2009a). AMPK is also known to interact with GSK-3β, and may be a link to the AKT/GSK-3β activation (Greco et al., 2008; 2009b). In the same way, in insulin resistance condition, the inhibition of insulin signal propagation inactivates AKT, impeding the signaling cascade to GSK3-β phosphorylation and activating tau phosphorylation (Schubert et al., 2003, 2004).

Berry fruits contain (poly)phenols such as flavonoids and anthocyanins involved in several mechanisms related to prevention of insulin resistance, obesity and cognitive impairment (Guo et al., 2012). The anthocyanins and metabolites have been shown to enter the brain, and their concentrations could be correlated with improved cognitive performance (Gutierres et al., 2014; Qin et al., 2013; Youdim et

al., 2003). In aged animals, the intake of polyphenol-rich fruits was able to reverse or delay age-related impairment when cognitive test challenges were performed (Malin et al., 2011; Shukitt-Hale et al., 2017). Recent studies indicate that diets supplemented with blueberries and jaboticaba (a Brazilian berry), for example, can prevent some of the neuronal deleterious effects caused by high-fat diet consumption (Batista et al., 2017; Carey et al., 2017).

Açai is a black-purple berry fruit native from Brazil, extensively studied due to its nutritional, phytochemical composition and impressive antioxidant activity and anti-inflammatory effects (De Oliveira et al., 2015; Dias et al., 2015; Schaus et al., 2006a). These properties were responsible for açai's high popularity in North America and European countries, where is known as “super-fruit”. Açai contains a variety of polyphenols, mainly anthocyanins and other flavonoids, responsible for its dark purple color and to its antioxidant activity. Furthermore, this berry is a great source of omega 9 (oleic acid) and dietary fibers (Smith et al., 2012; Luo et al., 2012; Pacheco-Palencia et al., 2009; Schauss et al., 2006) . In regards to this study, açai was once related to protection of brain cultured cells against induced oxidative stress and inflammatory insults in regions involved in overall brain function, memory and cognition, and in reversing the detrimental effects of aging on motor and cognitive behavior in aged animals (Carey et al., 2017).

These data led us to investigate the effect of açai pulp intake in the prevention of memory and cognitive impairment resulted of a high-fat diet intake, as well as to investigate hippocampal markers associated with tau phosphorylation. Furthermore, peripheral insulin resistance and markers of oxidative stress were explored in this study.

2. Material and methods

2.1 Açai pulp characterization

An Amazonian açai (*Euterpe sp*.) frozen pulp was obtained from a local market in Manaus-AM, Brazil. The açai pulp was freeze-dried to obtain a powder (AP). The analysis of macronutrients, total protein, moisture and ash was performed according to the methods described by the Association of Official Analytical Chemists

(AOAC, 2012). The total lipids were determined according to the Bligh & Dyer (1959) method, the total and insoluble dietary fibers were determined through the enzymatic-gravimetric method, according to the AOAC (1995) and the soluble fibers were calculated by difference between both. The concentration of carbohydrates was obtained as the subtraction between 100 and the total percentage of the other components.

A hexane extract was made in order to obtain a non-polar extract. For this, the AP samples were extracted by successive maceration with hexane at room temperature using a shaking water bath (170 rpm) for 1 hour at room temperature (22 °C ± 2). The solution obtained was then filtered, and extracted twice, combined and vacuum-concentrated in a rotary evaporator (T < 37 °C) to a final volume of 50 ml (0.1g mL^{-1}) (Prior et al., 2003). The extraction procedure was conducted in triplicate. The extract was then shaken in an Erlenmeyer flask containing isopropanol and hexane, then transferred to a separation funnel and gently washed with water, 3 times. The hexane extract was filtered in sodium sulfate and filled with hexane.

Using the same procedure as for the non-polar extraction, a polar fraction was obtained, extracting the remaining residue of açai pulp with successive maceration in 0.1% HCl in methanol in order to analyze the phenolic compounds. The solution obtained was then filtered, combined and vacuum-concentrated (T < 38 °C) to a final volume of 50 mL (0.1g mL^{-1}) (Degenhardt, Knapp, & Winterhalter, 2000; Kammerer, Carle, & Schieber, 2004).

2.1.1 Bioactive compounds

2.1.1.1 Non-polar bioactive compounds

To determine the total carotenoid content, the non-polar extract and blank (hexane) were read at 450 nm in the microplate reader and the calculation was made as described in a standard method (Higby, 1962).

The fatty acid composition was determined using an Agilent 6850 Series gas chromatography (GC) system (Agilent Technologies, Santa Clara, CA, USA) equipped with a capillary column. The samples were prepared following esterification according to a method described by Hartman & Lago (1973). Fatty acid methyl esters

were separated using AOCS method Ce 2-66 (AOCS, 2009), employing a 60-m DB-23 capillary column (50% cyanopropyl methylpolysiloxane; Agilent Technologies) with an internal diameter of 0.25 mm, coated with a 0.25 mm film. Chromatography was performed under the following conditions: heating at 110 ^{o}C for 5 min in an oven, followed by heating to 215 ^{o}C at a rate of 5 ^{o}C/min and holding at 215 ^{o}C for 24 min. The detector temperature was 280 ^{o}C, the injector temperature was 250 ^{o}C, helium was used as the carrier gas, the split ratio was 1:50, and the injection volume was 1.0 µL. The qualitative composition was determined via area normalization and was expressed as the mass percentage.

2.1.1.2 Polar bioactive compounds

Polar extract was diluted 100 times by pipetting 10 µL in 990 µL water. The liquid chromatographic – mass spectrometry (LC-MS) analysis was performed in negative ion mode using a High Performance Liquid Chromatography (HPLC, Hewlett Packard, Agilent Technologies 1290 series, City, country) coupled to a Q-ToF iFunnel 6550 mass spectrometer fitted with an electrospray ionization (ESI) source, using a Poroshell 120 SB-Aq 2.7 µm column (2.1x100 mm, Agilent). Mobile phase A was H2O containing 0.1% formic acid; and mobile phase B was acetonitrile containing 0.1% formic acid. The samples were eluted with a flow rate of 0.45 mL min^{-1} and the following linear gradient: 0 - 1 min, 5% B; 1 - 10 min, 5% B to 18% B; 10 -13 min 18% B to 70% B; 13 - 15 min, 70% B to 100% B; 15 - 17 min, 100% B and 3 min of post time at 5% B to column equilibration. The mass spectrometer parameters used were: VCap 3000 V; fragmentor voltage at 150 V; OCT 1RF Vpp at 750 V; Gas Temperature at 290 ^{o}C; Sheath Gas Temperature at 350 ^{o}C; drying gas at 12 L min^{-1}. Mass spectra were acquired in profile mode and the acquisition range was 100 - 1500 m/z. Data were treated using Agilent MassHunter Qualitative Analysis B 0.7 software.

The total phenolic content was determined by the Folin-Ciocalteu method, adapted from Swain & Hillis (1959). The absorbance was measured at 725 nm and the results were expressed as gallic acid equivalents (GAE mg g^{-1}).

The monomeric anthocyanins were quantified according to the pH-differential method described by Wrolstad (1976). The anthocyanin content was calculated as cyanidin 3-glucoside equivalent (C3G mg 100g^{-1}).

The flavonoids contents were determined by colorimetric reaction with aluminum chloride as described by Zhishen, Mengcheng, & Jianming (1999). Catechin was used as standard, the absorbance was read at 510 nm and the results were expressed as mg catechin g^{-1} (mg CE g^{-1}).

2.1.2 Antioxidant capacity

The H-ORAC (hydrophilic oxygen radical absorbance capacity) (Ou et al., 2013) test was carried out adding 20 µL of diluted methanolic extract or standard solutions, 125 µL of fluorescein diluted in phosphate buffer (pH 7.4), and 25 µL of AAPH (2,2-azobis (2-methylpropionamidine) dihydrochloride) to black microplates, in the dark. Trolox ((±)-6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid) was used as standard and a microplate reader with fluorescent filters: excitation wavelength, 485 nm; emission wavelength, 520 nm and the fluorescence was read each 1 min, for 80 min. The H-ORAC values were expressed in µmol trolox equivalent (µmol TE) per gram of AP by using the standard curves for each assay.

Lipophilic ORAC (L-ORAC) was based on the Ou et al., (2013) method. The non-polar extract of AP was diluted in a 7% randomly methylated β-cyclodextrin (RMCD) (RMCD Trappsol®) solution made in 50% aqueous acetone (v/v). After aliquoting appropriate diluted samples in dark microplates, fluorescein and AAPH diluted in phosphate buffer (7.4 pH) were quickly added to the plate. The microplate was read as in H-ORAC. The area under the curve was calculated as described in a previous work (Dávalos, Gómez-Cordovés, & Bartolomé, 2004). Values were expressed as µmol trolox equivalent (TE). The total-ORAC (T-ORAC) was assessed by the sum of H-ORAC values and L-ORAC values.

The ferric reducing ability of the methanolic extract was determined using the FRAP method (Rufino et al., 2010). The FRAP reagent was prepared in the dark, using 0.3 M acetate buffer (pH 3.6), 10 mM TPTZ in a 40 mM HCl solution and 20 mM $FeCl_3$ (at proportions of 10:1:1). The sample or standard solutions, water and FRAP reagent were mixed and incubated in an oven for 30 min at 37 °C. The samples and trolox standard curve were read at 595 nm.

These analyses were done in the microplate reader (Synergy HT, Biotek, Winooski, USA) spectrophotometer with Gen5™ 2.0 data analysis software.

2.2 Animal experimentation

This research followed the Animal Experimentation Ethical Principles adopted by the Brazilian Society of Laboratory Animal Science (SBCAL) and was approved by the Institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol #4212-1/2016). Fifty two 8-weeks old male Swiss mice (*Mus musculus*, weight 38.6 ± 2.9 g) obtained from the Multidisciplinary Center of Biological Investigation (CEMIB/UNICAMP) and fed with standard semi-purified diet AIN-93G (American Institute of Nutrition) (Reeves et al., 1993) were allocated in individual polypropylene cages, with free access to food and water, controlled relative humidity (60%–70%) and temperature (22 ± 2°C) and standard inverted 12/12 h dark/light cycles. The animals remained in acclimatization for 7 days receiving a semi-purified maintenance diet based on the AIN-93M (Reeves et al., 1993). Then they were randomly assigned into four groups (n = 13) according to the offered diet (Table 1) : a) normal-fat diet (AIN-93M) - N group; b) normal-fat diet with 2% AP (w/w) – NA group; c) high-fat diet (60% total calories from fat) – H group and d) high-fat diet containing 2% AP (w/w) - HA group. The dose of AP in the experimental diets were chosen based on its anthocyanin content according to previous studies using anthocyanin-rich berry (Batista et al., 2013, 2014). Furthermore, other research groups have also used 2% açai in the diet with the purpose to study metabolic diseases (Carey et al., 2017; Souza et al., 2010; Poulose et al., 2017). The diets were replaced at intervals of 2 days to prevent fat and anthocyanin oxidation. Food intake was monitored every 2 days and weight gain weekly.

Table 1. Composition of experimental diets

Ingredient (g kg $^{-1}$)	**N**	**NA**	**H**	**HA**
Casein (84,59% protein)	140.67	140.67	140.67	140.67
Corn starch	465.25	461.13*	264.99*	260.81*
Maltodextrin	154.85	153.48*	88.18*	86.80*
Sucrose	99.90	99.02*	56.89*	56.01*
Soy oil	40.00	31.60$^{\#}$	40.00	31.60$^{\#}$

Cellulose	50.00	44.77**	50.00	44.77**
Lard	-	-	310.00	310.00
Mineral mix	35.00	35.00	35.00	35.00
Vitaminic mix	10.00	10.00	10.00	10.00
L-Cystine	1.80	1.80	1.80	1.80
Choline bitartrate	2.50	2.50	2.50	2.50
tert-Butylhydroquinone	0.008	0.008	0.008	0.008
Açai pulp	-	20.00	-	20.00
Calories[a]	3717.22 ± 28.67	3695.28 ± 52.10	5294.92 ± 21.51	5238.02 ± 79.76

N: normal-fat diet fed group; NA: normal-fat diet plus 2% AP group; H: high-fat diet fed group; HA: high-fat diet containing 2% AP group. *The carbohydrate ingredients were adapted according to the lard content and/or the addition of freeze-dried açai pulp (AP) powder. [#]The soy oil content was adapted and discounted according to the amount of total lipids of AP. **The cellulose content was adapted and discounted according to the amount of total dietary fibers of AP. [a]Calories values (kcal kg^{-1}) were calculated considering the conversion factors of Atwater.

At the end of the 10th week, the animals were euthanized after injection of ketamine chloride (100 mg kg^{-1}) and xylazine chloride (10 mg kg^{-1}) in a concentration of 2:1 (v v^{-1}). After euthanizing, brains were rapidly removed, washed in a 0.9% saline solution, weighed, frozen in liquid nitrogen and kept at -80°C until posterior analyses.

2.2.1 Novel Object Recognition test

Non-spatial declarative memory was assessed in novel object recognition test (NOR) at the 8th week of experiment (*n* =10). The NOR apparatus was located in a separate dark room containing two red-light lamp bulbs. A video camera was suspended above the center of the arena, and the image was projected to a monitor to allow the experimenter to observe the animal's behavior in an adjacent room. The

apparatus used for the test was an open field arena (30 cm × 30 cm × 40 cm) made of coated wood. The walls were white and the floor was black. The objects used were a tower of Lego® bricks and a Falcon tube filled with colored sand. All objects were fixed to the floor with velcro, 15 cm apart and 5 cm from the wall. The test began between 9:00 and 10:00 a.m. and was divided in three phases: during the habituation phase, mice individually explored the open field freely for five minutes. In the familiarization phase, two identical objects were placed diagonally in the open field. The mice were placed in the arena facing away from the objects and were allowed to explore the objects for five minutes. Twenty-four hours later (during the test phase) the mice was introduced into the field again. In this phase, two objects were placed at the same position as in the familiarization phase and one of the familiar objects was replaced with a novel object. The animals were allowed to explore freely both the objects for five minutes. The positions of objects in the test phase were counterbalanced between animals to avoid positional biases. After each phase session, the animals were returned to their home cages and the arena and the objects were cleaned with 70% ethanol. All the test phases were recorded and later analyzed using stopwatches by blinded observers. Exploration was defined as direct contact of the nose or front paws with the object. Time spent exploring each object during the test phase was calculated as a recognition index (RI): a percent of time spent exploring the familiar (T_F) or the novel object (T_N) relative to the total time spent exploring both objects [RI = T_N or $T_F/(T_N + T_F)$] (Carey et al., 2009). The NOR task evaluates the rodent's ability to recognize a novel object in the environment. Since the NOR's methodology assesses the rodent's natural preference for novel objects, a higher percentage of time spent with the novel object is considered an index of improvement in learning and memory performance using the hippocampus (Carey et al., 2009).

2.2.2 Glucose Tolerance Test and Insulin Tolerance Test

A glucose tolerance test (GTT) was performed in 6 h-fasted mice in the 9th week of experiment by intraperitoneally (ip) injecting a solution of D-glucose (2 g kg^{-1}). Blood glucose levels were measured with an FreeStyle Lite glucometer (Abbott Diabetes Care, Alameda, CA, USA) using appropriate test strips. The venous blood

was collected via tail vein before (0 min) and 30, 60, 90 and 120 min after glucose injection.

The insulin tolerance test (ITT) was carried out in 6 h-fasted mice on the 10th week of experiment. The blood sample was collected via tail vein and the glucose levels were measured before (0 min) and 15, 30, 45 and 60 min after an ip-injection of saline solution (0.9%) containing 0.75 units kg^{-1} insulin (Novolin R, Novo Nordisk Bagsvaerd, DK).

In the last day of experiment (70th day) the fasting (6 h) blood glucose was also measured via tail vein using the glucometer.

2.2.3 Adiponectin

Fasting (6 h) serum adiponectin was assessed using a specific ELISA kit (Cat. #EZMADP-60K, Millipore, St. Charles, Missouri, USA).

2.2.4 Antioxidant enzyme activities

The hippocampus dissection was performed as described in a previous study (Batista et al., 2017). Approximately 100 mg mL^{-1} of tissue were homogenized in phosphate buffer (PB), pH 7.4 and the homogenates were used for antioxidant status analyses described below. The protein concentrations of the tissue homogenates were determined using the Bradford method (Bradford, 1976).

The measurement of reduced glutathione (GSH) and superoxide dismutase (SOD) values in tissue homogenates and serum was conducted as detailed in a previous study (Batista et al., 2014). For catalase (CAT) activity, we performed the methodology described in Batista et al (2017).

2.2.5 Western Blotting

Western blotting was performed in hippocampal homogenates (n = 5/ group). The dissected hippocampi were homogenized in a protein extraction cocktail (10 mmol L^{-1} ethylenediamine tetraacetic acid, 2 mmol L^{-1} phenylmethane sulfonyl-fluoride, 100 mmol L^{-1} NaF, 10 mmol L^{-1} sodium pyrophosphate, 10 mmol L^{-1} NaVO4, 10 µg mL^{-1} aprotinin and 100 mmol L^{-1} Tris, pH 7.4). Cellular protein was quantified by Bradford assay (Bio-Rad, Hercules, CA, USA), then 40 µg of the cleared lysates

were separated on 8% (AKT, GSK3-β and SYP) or 12% (tau) SDS-PAGE and electrotransferred onto nitrocellulose membrane (BioRad). After electrotransfer, the membranes were prior incubated with 5% bovine serum albumin to block non-specific antigenic sites followed by washing with TBS-T (0.1% Tris-buffered saline with 0.05% Tween 20, pH 7.4). Subsequently, the membranes were incubated with primary antibodies overnight to assess the protein level of: p-AKT 1/2/3 (Ser 473)-R, p-GSK-3β (Ser 9), p-tau (Thr 205), SYP (D-4), IRS-1 (E-12), AKT 1/2/3 (H-136), GSK-3β (H-76), tau (TAU-5) from Santa Cruz Biotechnology (Santa Cruz, CA, USA). A molecular-weight standard was used and ran concurrently on each gel for accurate determination of the proper molecular weight for each antibody (BioRad #1610374). Then, the membranes were washed with TBS-T and incubated with appropriate secondary antibody. The β-actin antibody (Sigma-Aldrich) was used as internal control and the results were expressed as a ratio of p-proteins/total proteins in case of the phosphorylated ones or normalized by β-actin in case of synaptophysin (SYP). Membranes were exposed to chemiluminescence solution (Super Signal West Pico Chemiluminescent Substrate; Pierce Biotechnology, Rockford, IL, USA) for 3 min to detect reactive bands, and captured using Gene Gnome equipment and the GeneSys image acquisition software (Syngene Bio Imaging, Cambridge, UK). Blot band densitometry was acquired using the NIH ImageJ 1.45s software (Wayne Rasband, NIH, Bethesda, MD, USA).

2.3 Statistics

GraphPad Prism 5.0 (GraphPad Software, Inc. La Jolla, CA, USA) was employed for statistical analysis. Weekly weight gain, GTT and ITT tests were analyzed using two-way ANOVA followed by Bonferroni test ($p < 0.05$). The Student's *t* test ($p < 0.05$) was used to compare group pairs according to the diet relations (N × H; N × NA and H × HA).

3. Results

3.1 Chemical characterization of açai pulp

The results of macronutrients composition, bioactive compounds and antioxidant capacity of freezed-dried açai pulp are displayed in Table 2. The AP

showed high energy content (598.38 kcal $100g^{-1}$) mainly due to its fat content which corresponded to 63.18% of the calories contained in the pulp, whereas only 31.5% are equivalent to the energy coming from the carbohydrates. The total dietary fiber correspond to 27,36% of the pulp.

The AP powder contains functional compounds of interest and have shown great antioxidant capacity as observed in the Folin–Ciocalteau, total flavonoids, monomeric anthocyanins, FRAP and H-ORAC methods (Table 3). In addition, some phenolic compounds were identified in the AP by LC-MS analysis, such as cyanidin-3-rutinoside, catechin and cyanidin-3-glucoside (Table 3).

Regarding non-polar compounds, fatty acids, carotenoids and L-ORAC was assessed demonstrating the major fatty acids in AP as oleic, palmitic, linoleic and stearic acids by CG analysis and interesting amounts of carotenoids corroborating to the total antioxidant capacity of açai (Table 4).

Table 2. Macronutrients, phenolics and antioxidants of açai pulp*.

Component	**Value****
Moisture (g 100 g^{-1})	0.97 ± 0.04
Protein (N x 6,25) (g 100 g^{-1})	7.85 ± 0.21
Lipids (g 100 g^{-1})	42.01 ± 0.95
Ash (g 100 g^{-1})	1.95 ± 0.09
Total carbohydrates (g 100 g^{-1})	47.20 ± 0.80
Total dietary fibers (g 100 g^{-1})	27.36 ± 0.79
Soluble dietary fibers (g 100 g^{-1})	8.75 ± 0.13
Insoluble dietary fibers (g 100 g^{-1})	18.61 ± 0.46
Total (poly)phenols (mg 100 g^{-1})	3906.87 ± 180.16
Total flavonoids (mg 100 g^{-1})	2365.84 ± 82.25
Monomeric anthocyanins (mg 100 g^{-1})	1573.45 ± 28.29
Total carotenoids (mg 100 g^{-1})	5.53 ± 0.007

Antioxidant capacity	
H-ORAC (µmol TE g^{-1})	664.16 ± 15.66
L-ORAC (µmol TE g^{-1})	7.71 ± 0.24
T_ORAC (µmol TE g^{-1})	671.88 ± 15.68
FRAP (µmol TE g^{-1})	407.44 ± 5.38

*Freeze-dried samples. **Expressed as wet weight of samples.

Table 3. Phenolic compounds identified in AP methanolic extract using HPLC-ESI(−)-MS/MS.

#	Name	Formula	Precursor	RT	RA (%)	MS/MS
1	Unknown	$C_{21}H_{24}O_{11}$	451.122	0.63	1.19	304/174
2	Citric acid	$C_6H_8O_7$	191.020	0.784	2.16	158/103
3	(Homo)2-citrate	$C_8H_{12}O_7$	219.051	1.524	2.41	201/166/73
4	Dihydroxybenzoic acid	$C_7H_6O_4$	153.019	1.759	4.83	137/123/119/69
5	Catechin-*O*-β-D-dlucopyranoside	$C_{21}H_{24}O_{11}$	451.125	3.491	1.84	289/137/271
6	Catechin[#]	$C_{15}H_{14}O_6$	289.072	3.875	8.99	289/245/151/123/109
7	Unknown	$C_{27}H_{32}O_{16}$	611.162	4.249	0.13	-
8	Kaempferol-*O*-3?-rutinoside	$C_{27}H_{30}O_{15}$	593.150	5.228	0.2	593/430/284
9	Epicatechin[#]	$C_{15}H_{14}O_6$	289.071	5.24	0.74	289/174/109
10	Unknown	$C_{21}H_{22}O_{11}$	449.108	5.263	0.12	-
11	Cyanidin-O-rutinoside hydration*	$C_{27}H_{32}O_{16}$	611.164	5.771	9.61	611/475/285/149
12	Cyanidin-*O*-glucoside	$C_{21}H_{20}O_{11}$	447.094	6.023	2.14	-
13	Cyanidin-*O*-rutinoside hydration*	$C_{27}H_{32}O_{16}$	611.162	6.563	14.14	611/475/285

14	Cyanidin-*O*-rutinoside	$C_{27}H_{30}O_{15}$	593.1528	6.566	31.96	593/284
15	Desconhecido	$C_{21}H_{22}O_{10}$	433.115	6.573	2.49	-
16	Apigenin-C-diglycoside	$C_{27}H_{30}O_{15}$	593.1507	7.405	0.24	593/473/353/269
17	Citrusin B	$C_{27}H_{36}O_{13}$	613.2153	7.706	0.11	-
18	Isolariciresinol monoglucoside isomer 1	$C_{21}H_{22}O_{11}$	521.2033	7.904	0.44	567/521/359
19	Apigenin derivative	$C_{21}H_{22}O_{11}$	449.1097	8.25	6.01	449/269/174/151
20	Citrusin B	$C_{27}H_{36}O_{13}$	613.2145	8.284	0.09	-
21	Isolariciresinol monoglucoside isomer 2	$C_{21}H_{22}O_{11}$	521.2032	8.543	7.56	567/521/359
22	Luteolin C-glucoside	$C_{21}H_{20}O_{11}$	447.0936	8.551	0.99	447/357/327/298
23	Isolariciresinol monoglucoside isomer 3	$C_{21}H_{22}O_{11}$	521.2033	8.729	0.37	567/521/359
24	Apigenin derivative	$C_{21}H_{22}O_{11}$	449.1091	9.217	0.62	449/269/151
25	Luteolin O-glucoside	$C_{21}H_{20}O_{11}$	447.0942	10.497	0.33	447/285/284
26	Unknown	$C_{21}H_{22}O_{11}$	567.2092	10.63	0.13	-
27	Luteolin[#]	$C_{15}H_{10}O_6$	285.0411	12.184	0.15	-

RT: retention time; RA: relative abundance. *Solvent derived artifacts due presence of water with flavylium cation (anthocyanins). [#] Identified with analytical standards.

Table 4. Fatty Acids in freezed-dried Açai pulp.

Fatty Acid	**Formula**	**%**
Saturated Fatty Acid		
Myristic	14:0	0.087
Palmitic	16:0	17.349
Margaric	17:0	0.077

Stearic	18:0	3.103
Eicosanoic	20:0	0.312
Behenic	22:0	0.052
Lignoceric	24:0	0.059
Total		*21.039*
Monounsaturated Fatty Acid		
Palmitoleic	16:1	0.374
Margaroleic	17:1	0.071
Oleic	18:1	69.519
Gadoleic	20:1	0.158
Total		*70.122*
Polyunsaturated Fatty Acids		
Linoleic	18:2	8.039
Linolenic	18:3	0.793
Total		*8.832*

3.2 Mice's food and energy intake, body and tissue weights

The animals fed the high-fat diets showed higher body weights from the fourth experimental week ($p < 0.01$). The NA group had lower body weight gain ($p < 0.05$) than the control group (N) (Table 5), even though the total food and calorie intake were similar between these groups (Table 5). The mice fed the normal-fat diets had a higher food intake in comparison to the high-fat diets-fed groups, but the opposite was observed concerning the total energy intake (Table 5).

Regarding to the adipose tissue pad weight (EAT, RAT and MAT), the intake of the high-fat diet increased the fat mass in H group in comparison to N (Table 5). Although, even if the body weight was not different ($p < 0.05$) between H and HA groups, the EAT and MAT weights were smaller in the HA-mice, when compared to H (23.6% and 19.9% reduction, respectively) but no differences were found for RAT weight. The relative brain weight was lower in the high-fat diet-fed mice than the normal-fat group. No changes were observed in the relative liver weight.

Table 5. Growth, food intake parameters and tissue weights ratio (g 100 g^{-1} total body weight) of the mice after 10 experimental weeks.

Parameter	N	NA	H	HA
Final body weight	52.56 ± 4.45	48.54 ± 4.48$^{\bullet}$	59.85 ± 4.92$^{\#\#\#}$	57.97 ± 4.55
Total weight gain	14.01 ± 3.13	12.31 ± 2.22$^{\bullet}$	20.46 ± 3.42$^{\#\#\#}$	19.12 ± 4.04
Food intake	407.23 ± 35.87	384.55 ± 37.28	293.22 ± 39.65$^{\#\#\#}$	282.87 ± 70.71
Calorie intake	1487.34 ± 112.71	1400.88 ± 122.13	1601.26 ± 151.10$^{\#}$	1535.47 ± 383.17
Brain	0.657 ± 0.020	0.704 ± 0.014	0.591 ± 0.017$^{\#}$	0.579 ± 0.010
Liver	4.632 ± 0.236	4.299 ± 0.131	5.159 ± 0.421	4.854 ± 0.266
EAT	4.312 ± 0.17	4.534 ± 0.26	5.370 ± 0.31$^{\#\#}$	4.104 ± 0.25**
RAT	1.527 ± 0.09	1.764 ± 0.13	2.530 ± 0.38$^{\#}$	2.446 ± 0.23
MAT	1.791 ± 0.09	1.678 ± 0.12	2.879 ± 0.16$^{\#\#\#}$	2.361 ± 0.13*

N: normal-fat diet fed group; NA: normal-fat diet plus 2% FAP group; H: high-fat diet-fed group; HA: high-fat diet containing 2% FAP group. Final body weight, total weight gain and food intake (g); Calorie intake (kcal); EAT: epididymal adipose tissue; RAT: retroperitoneal adipose tissue; MAT: mesenteric adipose tissue. Values are expressed as mean ± SEM, n = 13. Data were analyzed using analysis of variance followed by Student's t test. $^{\#}$Indicates significant differences between groups N and H; $^{\bullet}$Indicates significant differences between groups N and NA; *Indicates significant differences between groups H and HA according to Student's t test $^{\bullet}$, $^{\#}$ or *$p < 0.05$; $^{\#\#}$ or **$p < 0.01$; and $^{\#\#\#} < 0.001$.

3.3 Glucose Tolerance Test, peripheral insulin resistance and fasting adiponectin

In the GTT and ITT tests, the H group showed the highest ($p < 0.05$) AUC in response to both glucose and insulin injection, when compared to the N group (Figure 1). The AUC values for blood glucose levels during the GTT did not differ between the high-fat diet-fed groups, which demonstrate that glucose intolerance was not prevented by the intake of HA-diet. However, the blood glucose response after insulin injection in ITT was lower in HA versus H, suggesting a better insulin sensitivity in the HA-fed animals (Figure 1). Furthermore, fasting glucose and fasting adiponectin in the last experimental day was higher in H in comparison to N and HA groups ($p < 0.05$; Figure 2).

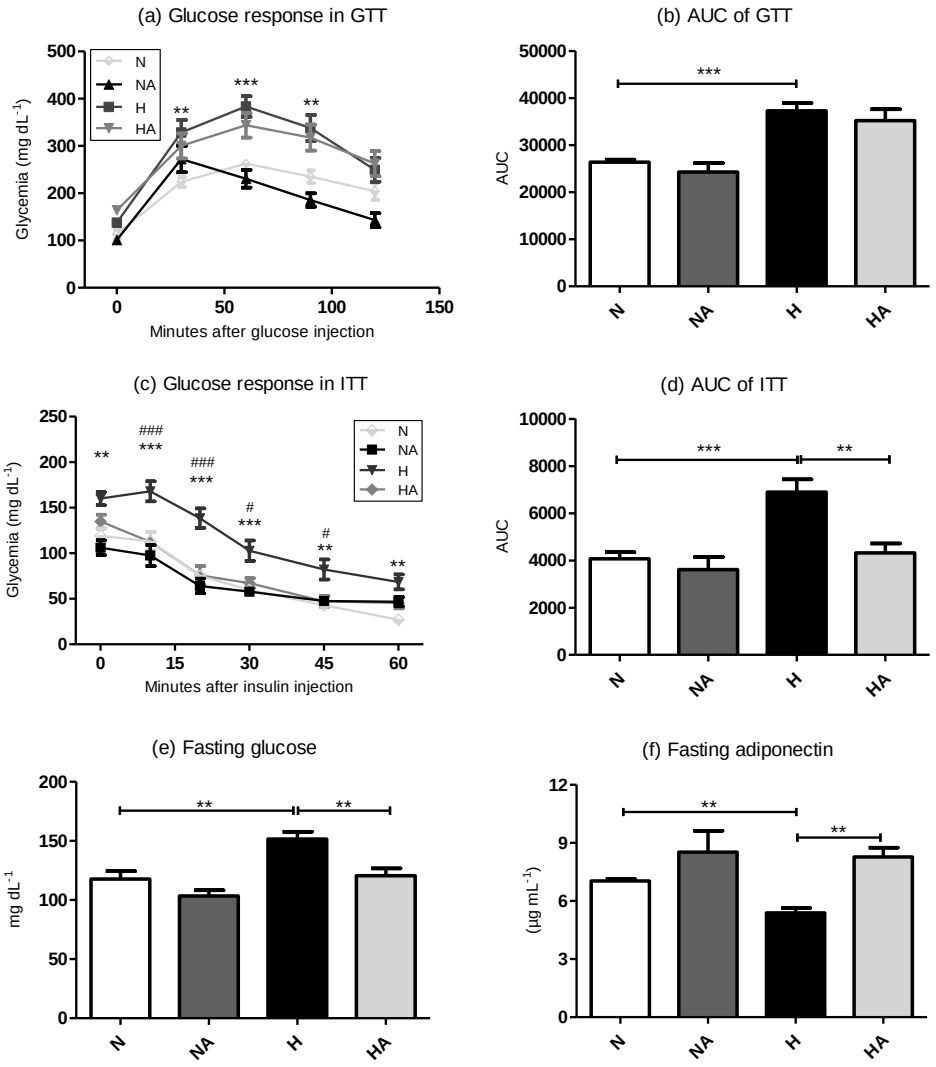

Figure 1 - Glycemic response in GTT test (a); AUC response in GTT test (b); Glycemic response in ITT test (c); AUC response in ITT test (d); Fasting blood glucose levels (e). N: normal-fat diet-fed group; NA: normal-fat diet plus 2% FAP group; H: high-fat diet fed group; HA: high-fat diet containing 2% FAP group. In (a) and (c) data were analyzed by two-way ANOVA and the Bonferroni *post-hoc* test. In (b), (d) and (e) the Student's *t* test was applied. * Indicates difference between N and H groups and $^{\#}$indicates difference between H and HA groups. * and $^{\#}$ Indicates $p < 0.05$, ** $p < 0.01$ and *** and $^{\#\#\#}$ $p < 0.001$, $n = 6$.

3.4 Antioxidant defense

The hippocampal and serum catalase activity in the high-fat diet-fed mice were lower than those fed the normal-fat diet (Table 6). However, the addition of AP in high-fat diet seemed to play a protective role in the hippocampus and serum showing an improvement in the CAT activity (Table 6). Nevertheless, the AP-supplemented groups showed an increase in hippocampal and serum (NA) levels, when compared to their respective control groups. The SOD activity was lower in H group in comparison to the normal fat diet-fed group and the addition of AP in this diet improved the antioxidant activity of this enzyme in NA group. GSH hippocampus HA H

Table 6. Antioxidant enzyme activity and reduced glutathione contents in the mice groups after 10 experimental weeks.

Enzyme	N	NA	H	HA
		Hippocampus		
CAT	3.62 ± 0.13	4.05 ± 0.32	2.80 ± 0.31$^{\#\#}$	3.82 ± 0.60*
GSH	174.23 ± 11.67	219.11 ± 16.77	158.03 ± 19.68	199.41 ± 26.74
SOD	4.54 ± 0.12	5.39 ± 0.18*	4.52 ± 0.48	4.28 ± 0.24
		Serum		
CAT	19.53 ± 1.73	19.58 ± 1.10	14.94 ± 1.34$^{\#}$	21.00 ± 1.78*
GSH	133.27 ± 2.41	128.74 ± 6.19	118.68 ± 6.89$^{\#}$	138.70 ± 6.41
SOD	19.42 ± 1.32	18.85 ± 0.76	14.25 ± 1.51$^{\#}$	15.74 ± 0.95

N: normal-fat diet fed group; NA: normal-fat diet plus 2% AP group; H: high-fat diet fed group; HA: high-fat diet containing 2% AP group. CAT: catalase (nmol min^{-1} mg^{-1} formed formaldehyde); GSH: reduced glutathione (nmol mg^{-1}); SOD: superoxide dismutase (U mg^{-1}); $^{\#}$Indicates significant differences between groups N and H; *Indicates significant differences between groups H and HA according to Student's *t* test $^{\#}$ or *$p < 0.05$ and $^{\#\#}$ $p < 0.01$. Values expressed as means ± SEM, $n = 5$.

3.5 Hippocampal protein quantification

The AKT/GSK3-β/tau axis was assessed in the hippocampus of the mice, a region of

the brain involved in learning and memory. The higher phosphorylation of AKT in the groups N and HA relative to the H group improved the phosphorylation and inactivation of GSK3-β in serine 9 in the HA group. A trend of lower phosphorylation of GSK3-β was observed in H group in comparison to N (p = 0.067). Tau phosphorylation in Thr 205 was increased in the hippocampus of H group when compared to N, indicating the deleterious effect of the diet. A trend of lower phosphorylation of tau in Thr 205 was observed in the group fed HA diet (p = 0.079) (Figure 2). No differences were observed in synaptophysin (SYP).

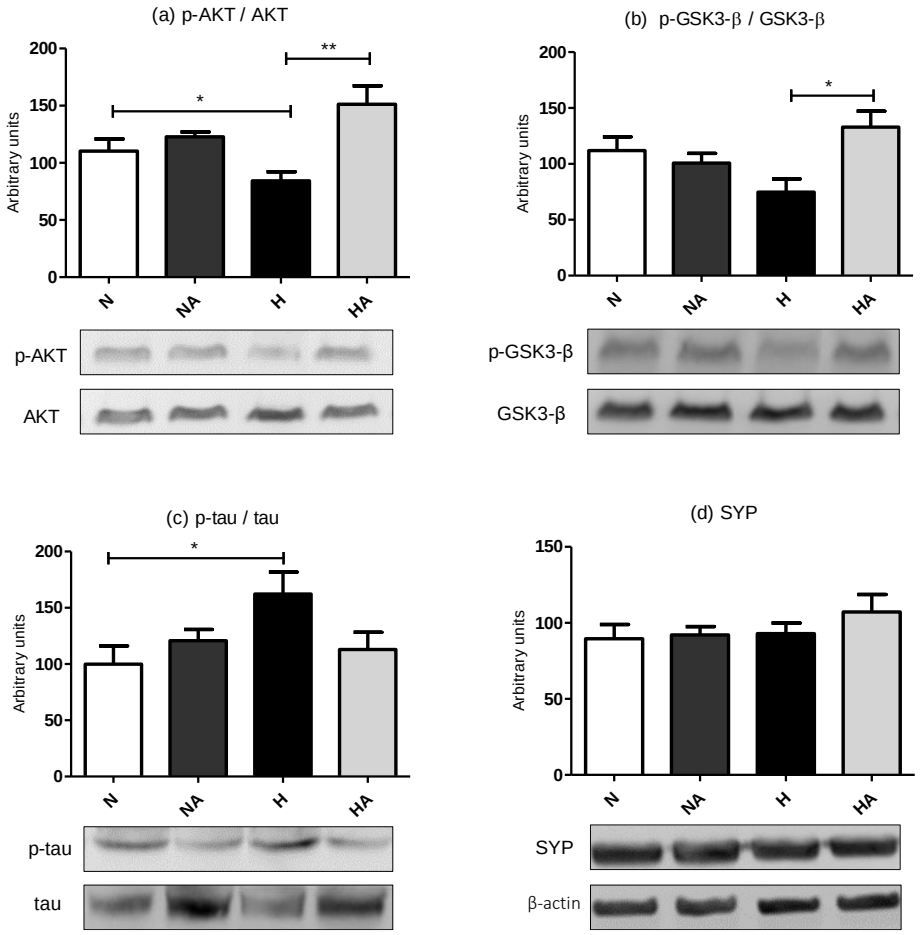

Figure 2 – AKT phosphorylation (a); GSK3-β phosphorylation (b); tau phosphorylation (c); synaptophysin (d). N: normal-fat diet fed group; NA: normal-fat diet plus 2% AP group; H: high-fat diet fed group; HA: high-fat diet containing 2% AP group. The results are shown as a percentage of the control (N group as 100%) Data were analyzed using analysis of variance followed by Students *t* test. *Indicates $p < 0.05$ and **$p < 0.01$ between N × H and H × HA groups, $n = 5$.

3.6 Novel Object Recognition

The high-fat group demonstrated significantly decrease in recognition index (RI) when exposed to novel object compared to N group, failing to show a preference for the novel object, suggesting a deficit in object recognition memory in this test. Although, HA-mice showed preference for novel object over the familiar ones ($p < 0.05$), suggesting improvement in object recognition memory when compared to high-fat diet-fed mice (H) (Figure 3).

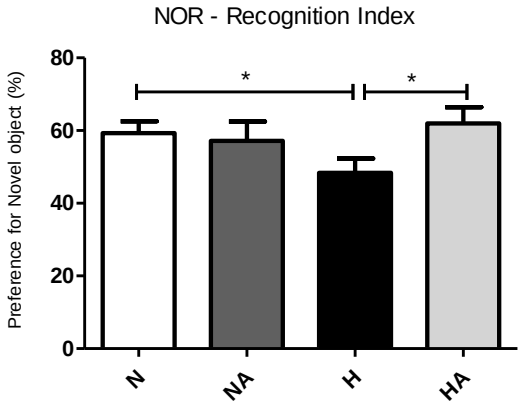

Figure 3 - Long-term recognition memory assessed by the novel object recognition (NOR) test**.** Recognition Index: a percent of time spent exploring the familiar or the novel object relative to the total time spent exploring both objects. N: normal-fat diet fed group; NA: normal-fat diet plus 2% FAP group; H: high-fat diet fed group; HA: high-fat diet containing 2% FAP group. *Indicates $p < 0.05$ according to analysis of variance followed by Student's *t* test.

4. Discussion

Many studies have demonstrated the effects of açai intake on health, highlighting anti-inflammatory, antioxidant, antitumoral and anti-obesogenic properties (Choi et al., 2017; De Oliveira et al., 2015; Dias et al., 2015; Schauss, Wu, Prior, Ou, Huang, et al., 2006; Silva et al., 2014). However, there are few studies relating açai intake effects on cognition. Since the past decade, obesity, marked by the consumption of a diet rich in saturated fatty acids and simple sugar, has been related as a risk factor for both insulin resistance and cognitive impairment (Beydoun, Beydoun, & Wang, 2008; Leboucher et al., 2013). In this study, we showed that the

addition of açai pulp to a high-fat diet prevented peripheral insulin resistance, visceral fat mass gain and improved cognitive behavior in mice.

Açai is known by its great amount of bioactive compounds like (poly)phenols, especially flavonoids and anthocyanins as predominant phytochemicals. Cyanidin 3-rutinoside, one of the identified compounds in this study, was cited as the major anthocyanin in açai (1.25 – 1.93 mg g^{-1}), followed by cyanidin-3-glucoside (0.95 – 1.17 mg g^{-1}) (Pacheco-Palencia, Duncan, & Talcott, 2009; Schauss et al., 2006). Both anthocyanins are extensively related to health benefits in other animals models, in vitro and also in human trials (Ereminas et al., 2017; Karlsen et al., 2007; Qin et al., 2009; Thilavech et al., 2017; You et al., 2017). Furthermore, we found monounsaturated fatty acid (MUFA) (oleic acid, omega-9) as the main fraction of the total lipids in açai pulp. Regarding dietary fiber, the insoluble fraction was the predominant. Also its content of sugars is very low (Schauss et al., 2006). These findings are similar to other studies that characterized açai; however it is possible to find some divergences into different species (Smith et al., 2012; Luo et al., 2012; Schauss et al., 2006).

Although açai supplementation did not prevent weight gain in the high-fat group, we observed a reduction in adipose tissue pad weights (MAT and EAT). The prevention of fat mass gain has also been observed after administration of 2% açai pulp in rodents being associated or not with total body weight loss (De Oliveira et al., 2015; Guerra et al., 2015). A previous report evaluated the consumption of different types of berries and showed increased total weight gain and liver weight in rodents after intake of 20% açai in a high-fat diet. However, the same study related açai intake to increased lean body mass percentage and the relative size of the epididymal fat pad was lower in group receiving açai, when compared to the high-fat group (Heyman et al., 2014). Açai was the berry who had the highest fat content among all the assessed berries in the mentioned study (Heyman et al., 2014), and the 20% supplementation represents a total of 3.46 g 100 g^{-1} in diet of palmitic acid in the diet. In this context, we chose smaller dose of açai, since an elevated consumption of palmitic acid was once related to increase of body weight and liver triglycerides in mice, which could explain their findings (Wit et al., 2012).

The adipose tissue is responsible for the production of adipokines such as leptin and adiponectin, but in obesity, due to the hypertrophy and consequent tissue

inflammation, the production of these cytokines is unbalanced (Tilg & Moschen, 2006; Zhang & Scarpace, 2006). Adiponectin has a potent insulin-sensitizing effect and in obesity both adiponectin release and adiponectin receptors are down-regulated. The up-regulation of this adipokine and adiponectin receptor, as well as its functions is one of the mechanism of most interest in terms of prevention of obesity-linked insulin resistance (Yadav et al., 2013). In our study, we showed a lower peripheral insulin resistance and elevated levels of adiponectin in serum of the mice that consumed the HA-diet. The C3G anthocyanin was once reported to stimulate the expression of adiponectin by the adipose tissue probably because its involvement in the acetylation of FoxO1, which is a transcription factor that regulates the adiponectin production (Liu et al., 2014).

A recent study has demonstrated that the treatment of obese mice with an açai aqueous extract (dose) also attenuated insulin resistance and ameliorated adiponectin levels in serum with no alteration the body weight gain (Carey, Miller, et al., 2017). Even that the whole pulp fruit, including the lipid fraction, was used in our experimental diets, our results corroborate the cited study, evidencing that the proportion of palmitic acid of the supplementation (0.34 g 100 g^{-1}) was not harmful according to the analyzed parameters. Furthermore, as we demonstrated, açai has oleic acid as the main fatty acid in its composition. Studies demonstrated that the addition of MUFAs to the diet of healthy subjects improved insulin sensitivity (Vessby et al., 2001). The consumption of MUFAs has also prevented postprandial decrease in peripheral adiponectin gene expression and insulin resistance in insulin-resistant subjects (Paniagua González et al., 2007). Thus, the fatty fraction might be the differential advantage from açai in relation to other berries.

Diets rich in fruits and their (poly)phenols have been used in many studies targeting the improvement of brain functions such as learning and memory (Jeon et al., 2012). Flavonoid and anthocyanin-rich foods were shown to be effective in reversing deficits in learning and memory, which could be affected by aging or a long-term consumption of high-fat diets (Batista et al., 2017; Carey et al., 2017; Carey et al., 2014). Our research group has demonstrated that the intake of a Brazilian berry concomitantly to a high-fat diet could prevent the impairment in hippocampal function (Batista et al., 2017). The present study extended these searches to açai pulp. We showed that the consumption of HA-diet increased the

phosphorylation of AKT, leading to a higher phosphorylation and consequent inactivation of GSK3-β and a trend to lower tau phosphorylation at the Thr 205 site in the hippocampus of the mice. In an insulin resistance status, caused by obesity, AKT's propagation signals is inactivated (Greco et al., 2009). The AKT kinase is responsible for phosphorylating GSK3-β, which when phosphorylated is inactive on phosphorylating tau, preventing subsequent formation of NFT, a classic hallmark of tauopathies observed in many neurodegenerative diseases (Kaytor & Orr, 2002). A higher activation of IRS in hippocampal tissue was associated to a reduced tau phosphorylation in the same model of mice fed a high-fat diet containing jaboticaba berry, which indicated a better central insulin sensitivity in those animals when compared to the control high-fat group (Batista et al., 2017; Schubert et al., 2003). This could be a possible mechanism to explain the increase of AKT phosphorylation in the HA group, since the insulin receptor can activate PI3K leading to phosphorylation and activation of AKT (Duan, Li, & Rui, 2004).

Many sites of phosphorylation of tau are been under recent research to discover the mechanisms of tauopathies . Tau binds to microtubules which provides a physical pathway for axonal transporters and support cellular structure in neuronal cells, and is regulated by many different kinases and phosphatases (Himmler et al., 1989). Its hyperphosphorylation compromises the capability to bind and to stabilize microtubules, as well as to aggregate when the equilibrium is harmed, resulting in the formation of higher order structures, such as NFTs (Köpke et al., 1993). In the present study, we evaluated the Thr 205 site and showed no significant decrease in phosphorylation of supplemented HA-group compared to high-fat group. However, the inactivation of GSK3-β could prevent tau's phosphorylation in other sites such as Ser 199, Ser 202, Ser 396, Thr 231, Thr 181, among others documented elsewhere (García-Font et al., 2016; Liang et al., 2016; Sawmiller et al., 2016; Song et al., 2016).

Although tau's phosphorylation did not decrease, the group fed the high-fat diet with açai pulp had a better behavior in NOR test relative to its control, presenting an improvement in object recognition memory, denunciating a prevention of cognitive impairment induced by the diet. The hippocampus plays a key role in comparing previously stored information with new incoming aspects of one particular situation, and is important for recognition memory as a "novelty detector"; if there are lesions in

this structure, it will occur moderate and reliable memory impairment (Broadbent et al., 2010; Clarke et al., 2010).

The better performance in NOR test by the mice fed the high-fat diet supplemented with açai might also have been related to other mechanisms. For example, the expression of neurotrophins like brain-derived neurotrophic factor (BDNF) through the ERK/CREB signaling were associated to the consumption of berries and flavonoids (Rendeiro et al., 2014; Williams et al., 2008). Studies have suggested that flavonoids induces both ERK1/2 and CREB activation (Maher, Akaishi, & Abe, 2006; Schroeter et al., 2007). The CREB's activation, in its turn, is involved with the expression of genes, such as BDNF (Tao et al., 1998). This neurothrophin appears to regulate neuronal function and memory (via the PI3 kinase/AKT/mTOR signaling pathway), leading to an increase translational efficacy and capacity (Bekinschtein et al., 2007; Wullschleger, Loewith, & Hall, 2006).

Previous data showed açai's administration efficacy in protect brain cell culture from oxidative stress and inflammatory insults in critical neuronal regions involved in memory, cognition and overall brain function (Carey et al., 2017). In a different model of cognitive/memory impairment, the consumption of açai has reversed the deleterious effects of aging on motor and cognitive behavior, as well as improved working memory assessed by the Morris water maze (a test of spatial memory) (Carey et al., 2017; Poulose et al., 2017). However, this is the first report about how the intake of açai pulp behaves in the prevention of hippocampal impairment induced by a high-fat diet in middle-aged mice investigating the tau phosphorylation and the NOR test.

We have also shown an elevated activity of CAT and a trend of increase in GSH values in the hippocampus of the HA group in comparison to the H group, indicating increase of the activation of the local antioxidant defense system. The CNS is especially vulnerable to free radical damage because of its lipid content. The brain also have a high oxygen consumption rate and a lower relative amount of antioxidant enzymes compared with other tissues (Coyle & Puttfarcken, 1993). An increased brain oxidative stress seems to have an important role in cognitive impairment caused by normal aging and neurodegenerative diseases (Padurariu et al., 2013) and the administration of diets containing components like (poly)phenols and other antioxidant compounds has been shown to improve such deficit, since the antioxidant

enzymes play a role in protection of cells against the oxidative stress (Batista et al., 2017; Veberic et al., 2015).

In conclusion, our results indicate that the supplementation of the high-fat diet with 2% açai pulp showed beneficial effects in prevention of the deleterious effects in peripheral insulin resistance and in cognitive impairment related to obesity. The improvement in AKT and GSK3-β phosphorylation seen in this group corroborates these findings and suggests more investigation of other protein signaling pathways in the hippocampus, like the CREB/BDNF, as well as other phosphorylation sites of tau. Thus, the cognitive improvements seen in NOR test by the animals that received the high-fat diet supplemented with açai pulp could be better elucidated.

Acknowledgements

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CAPÍTULO 3

CONCLUSÃO GERAL

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A polpa de açaí tem ganhado cada vez mais espaço na alimentação da população brasileira. Sua composição, rica em compostos bioativos como os polifenóis, antocianinas, flavonoides e carotenoides, lhe garantem alta capacidade antioxidante, que vem sendo extensivamente explorada na literatura em diversos parâmetros de saúde. Entre seus atributos, esta fruta se destaca pela sua alta contribuição energética devido ao elevado teor de lipídios, principalmente ácidos graxos monoinsaturados ômega-9 e pela grande quantidade de fibras dietéticas, principalmente as de característica insolúvel.

Ao ser adicionada à dieta hiperlipídica de camundongos, a polpa liofilizada de açaí não induziu alteração no consumo de dieta e no peso dos animais, mas os depósitos de gordura visceral dos mesmos estavam diminuídos. A suplementação com PA promoveu menor glicemia de jejum e maior sensibilidade periférica à ação da insulina durante o teste de tolerância à insulina pelos animais alimentados com dieta hiperlipídica, não havendo alteração no teste de tolerância à glicose. Houve um aumento nos níveis séricos de adiponectina nestes animais, prevenindo o efeito deletério do excesso de gordura na dieta.

No hipocampo foi observado uma melhora na fosforilação de enzimas envolvidas com a fosforilação da tau, entretanto esta não apresentou resultado significativo no grupo HA. O teste de reconhecimento de objetos revelou que os mesmos animais tiveram um melhor desempenho de memória, indicando que os compostos do açaí podem ter tido uma ação independente da fosforilação da tau.

Em suma, o consumo de açaí juntamente com uma dieta rica em gordura foi capaz de atenuar alguns efeitos deletérios à saúde e não promoveu nenhum efeito negativo à saúde, dentre os parâmetros estudados. Os resultados obtidos instigam novas pesquisas envolvendo outras vias metabólicas para completa elucidação dos efeitos aqui encontrados e constatação do papel do açaí como alimento funcional.

CAPÍTULO 4

REFERÊNCIAS GERAL

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ANEXO 1

Autorização CEUA

UNICAMP

CEUA/UNICAMP

CERTIFICADO

Certificamos que a proposta intitulada **Efeito da suplementação de açaí em dieta hiperlipídica na resistência à insulina e outras doenças correlatas à obesidade**, registrada com o nº **4212-1**, sob a responsabilidade de **Prof. Dr. Mário Roberto Maróstica e Nathalia Medina Dos Santos**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto o homem) para fins de pesquisa científica (ou ensino), encontra-se de acordo com os preceitos da **LEI Nº 11.794, DE 8 DE OUTUBRO DE 2008**, que estabelece procedimentos para o uso científico de animais, do **DECRETO Nº 6.899, DE 15 DE JULHO DE 2009**, e com as normas editadas pelo **Conselho Nacional de Controle da Experimentação Animal (CONCEA), tendo sido** aprovada pela **Comissão de Ética no Uso de Animais** da **Universidade Estadual de Campinas - CEUA/UNICAMP,** em **12 de maio de 2016**.

Finalidade:	**( ) Ensino (X) Pesquisa Científica**
Vigência do projeto:	**02/08/2016-29/11/2016**
Vigência da autorização para manipulação animal:	**02/08/2016-29/11/2016**
Espécie / linhagem/ raça:	**Camundongo heterogênico / Swiss**
No. de animais:	**52**
Peso / Idade:	**03 semanas / 35g**
Sexo:	**machos**
Origem:	**CEMIB/UNICAMP**

A aprovação pela CEUA/UNICAMP não dispensa autorização prévia junto ao **IBAMA**, **SISBIO** ou **CIBio**.

Campinas, 12 de maio de 2016.

Profa. Dra. Liana Maria Cardoso Verinaud
Presidente

Fátima Alonso
Secretária Executiva

IMPORTANTE: Pedimos atenção ao prazo para envio do relatório final de atividades referente a este protocolo: até 30 dias após o encerramento de sua vigência. O formulário encontra-se disponível na página da CEUA/UNICAMP, área do pesquisador responsável. A não apresentação de relatório no prazo estabelecido impedirá que novos protocolos sejam submetidos.

ANEXO 2

Comprovante de cadastro de acesso no SisGen (Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado)

Ministério do Meio Ambiente
CONSELHO DE GESTÃO DO PATRIMÔNIO GENÉTICO

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO

Comprovante de Cadastro de Acesso
Cadastro nº AEAAC6C

A atividade de acesso ao Patrimônio Genético, nos termos abaixo resumida, foi cadastrada no SisGen, em atendimento ao previsto na Lei nº 13.123/2015 e seus regulamentos.

Número do cadastro:	**AEAAC6C**
Usuário:	**Nathalia Medina dos Santos**
CPF/CNPJ:	**046.444.421-78**
Objeto do Acesso:	**Patrimônio Genético**
Finalidade do Acesso:	**Pesquisa**

Espécie

Euterpe oleracea

Título da Atividade:	**IMPACTO DO CONSUMO DE POLPA DE AÇAÍ PARA A PREVENÇÃO DO COMPROMETIMENTO COGNITIVO**

Equipe

Nathalia Medina dos Santos	**INDEPENDENTE**

Data do Cadastro:	**17/12/2017 03:19:46**
Situação do Cadastro:	**Concluído**

Conselho de Gestão do Patrimônio Genético
Situação cadastral conforme consulta ao SisGen em **18:39** de **17/12/2017**.

SISTEMA NACIONAL DE GESTÃO DO PATRIMÔNIO GENÉTICO E DO CONHECIMENTO TRADICIONAL ASSOCIADO - SISGEN