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FLÁVIA VITORINO FREITAS

“OBESIDADE ABDOMINAL E ESTRESSE EM POPULAÇÕES RURAL E URBANA: UMA ABORDAGEM EPIGENÉTICA”

Vitória, ES

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Biotecnologia, da Rede Nordeste de Biotecnologia, como parte dos requisitos para obtenção do título de Doutor em Biotecnologia.

Área de Concentração: Biotecnologia em Saúde

Orientadora: Prof. Dra. Adriana Madeira Alvares da Silva

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Área de Concentração: Biotecnologia em Saúde

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Vitória, ES, junho de 2018

Dedicatória

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Resumo

A hiperativação do eixo Hipotálamo-Pituitária-Adrenal está relacionada com o estresse psicossocial e com o acúmulo excessivo de tecido adiposo. O objetivo deste trabalho foi identificar indicadores de estresse psicossocial associados à adiposidade abdominal e avaliar a relação sobrepeso e metilação da região promotora do gene do receptor do glicocorticoide (NR3C1 1F região) em adultos. Este estudo transversal foi realizado com 384 indivíduos adultos (20 a 59 anos), usuários do Sistema Único de Saúde de uma cidade no Sudeste brasileiro. Utilizou-se como indicador de excesso de peso o Índice de Massa Corpórea (IMC) e a adiposidade abdominal (variável dependente) foi avaliada pelo perímetro da cintura. As variáveis independentes foram os indicadores de estresse psicossocial: Insegurança Alimentar e Nutricional, cortisol sérico, sintomas sugestivos de depressão pelo Beck Depression Inventory e pressão arterial alterada. Modelos de regressão linear univariada entre a adiposidade abdominal e cada indicador de estresse foram testados, estratificados por localização rural e urbana e, posteriormente, foram ajustados por variáveis socioeconômicas, de saúde e estilo de vida. Análises bioquímicas e moleculares foram realizadas com uma sub-amostra de 282 indivíduos agrupados quanto ao IMC (≥ e < 25 kg/m^2). Avaliou-se o perfil de metilação de NR3C1 1F região pelo método do pirosequenciamento. Por análise fatorial avaliou-se a inter-relação entre a metilação CpG sítio específico e extraiu-se os componentes principais, obtendo-se dois Bins de CpGs. Comparações de medianas, correlações de Spearman e regressão de Poisson com variância robusta foram utilizados para avaliar a associação entre excesso de peso e metilação de NR3C1 1F região. A prevalência de excesso de peso foi de 68,3% e 71,5% dos indivíduos apresentaram risco aumentado para complicações metabólicas relacionadas à adiposidade abdominal. Os indicadores de estresse que tiveram associação com a adiposidade abdominal foram: cortisol na população rural e pressão arterial alterada na população urbana. A sub-amostra não diferiu da amostra total quanto ao excesso de peso e às covariáveis. O grupo excesso de peso apresentou menores percentuais de metilação que o grupo sem excesso de peso nas análises do segmento total ($p < 0,05$), dos Bins de CpGs ($p < 0,05$) e dos CpGs sítio específicos: 41, 42, 44 e 45 (p-corrigido ≤ 0,037). A hipometilação no segmento total e no Bin 1 foi explicada pelo excesso de peso, quando controlado por covariáveis. Por fim, os resultados apontam para a influência do meio local e psicossocial na modulação do estresse uma vez a predição da adiposidade abdominal rural e urbana foi explicada por diferentes indicadores de estresse. Além disso, o excesso de peso foi relacionado com a hipometilação de NR3C1 1F região, estreitando a relação entre o estresse psicossocial e o acúmulo excessivo de gordura.

Abstract

Hyperactivation of the hypothalamic-pituitary-adrenal axis is related to psychosocial stress and excessive accumulation of adipose tissue. The objective of this study was to identify indicators of psychosocial stress associated with abdominal adiposity and to evaluate the weight excess and methylation relationship of the glucocorticoid receptor gene promoter region (NR3C1 1F region) in adults. This cross-sectional study was performed with 384 adult subjects (20 to 59 years), users of the Unified Health System of a city in the Southeast of Brazil. The Body Mass Index (BMI) was used as an indicator of weight excess and abdominal adiposity (dependent variable) was assessed by the waist circumference. The independent variables were the indicators of psychosocial stress: Food and Nutritional Insecurity, serum cortisol, symptoms suggestive of depression by Beck Depression Inventory and altered blood pressure. Univariate linear regression models between abdominal adiposity and each indicator of stress were tested, stratified by rural and urban location, and later were adjusted for socioeconomic, health and lifestyle variables. Biochemical and molecular analyzes were performed with a sub-sample of 282 individuals grouped for BMI (≥ and < 25 kg/m^2). The methylation profile of NR3C1 1F region was evaluated by the pyrosequencing method. By factorial analysis the inter-relationship between the specific CpG site methylation was evaluated and the main components were extracted, obtaining two Bins of CpGs. Comparisons of medians, Spearman correlations and Poisson regression with robust variance were used to evaluate the association between weight excess and methylation of NR3C1 1F region. The prevalence of weight excess was 68.3% and 71.5% of the subjects presented an increased risk for metabolic complications related to abdominal adiposity. The indicators of stress associated with abdominal adiposity were: cortisol in the rural population and altered blood pressure in the urban population. The sub-sample did not differ from the total sample for weight excess and covariates. The weight excess group had lower methylation percentages than the whithout weight excess group in the analyzes of the total segment ($p < 0.05$), the CpGs Bins ($p < 0.05$) and the specific CpGs site: 41, 42, 44 and 45 (p-corrected ≤ 0.037). Hypomethylation in the total segment and in Bin 1 was explained by the weight excess, when controlled by covariates. Finally, the results point to the influence of the local and psychosocial environment on the stress modulation once the prediction of rural and urban abdominal adiposity was explained by different indicators of stress. In addition, weight excess was related to hypomethylation of NR3C1 1F region, narrowing the relationship between psychosocial stress and excessive fat accumulation.

Sumário

1. Introdução

A obesidade é definida como acúmulo anormal ou excessivo de gordura que pode prejudicar a saúde (WHO, 2000). O excesso de peso acomete indivíduos de todas as idades e extratos sociais em todas as regiões do mundo e vem se configurando como uma epidemia global. A transição epidemiológica e demográfica, fundamentada em redução da taxa de mortalidade, maior expectativa de vida, envelhecimento populacional, rápida urbanização, desenvolvimento industrial e tecnológico e globalização, resulta em estilo de vida pouco saudável, com consequente aumento de prevalência de doenças crônicas. A obesidade, com seus desdobramentos de complicações à saúde, tem sido a principal causa de morbimortalidade (CHAN, 2013; RTVELADZE et al, 2013; WHO, 2018).

Por outro lado, as mudanças culturais, globalização e consequente aceleração do ritmo de vida também trouxeram aumento de doenças decorrentes de estresse psicossocial, levando preocupação às autoridades de saúde pública (GUNDERSEN et al, 2011; RAZZOLI & BARTOLOMUCCI, 2016). O estresse psicossocial ocorre quando as possibilidades de adaptação e resiliência aos agentes estressores são ultrapassadas e se estabelece a chamada fase de exaustão, o que ameaça a saúde e o bem estar individual ou mesmo coletivo (SELYE, 1950; FONSECA et al, 2009; SINHA & JASTREBOFF, 2013; ISASI et al, 2015).

A relação entre acúmulo excessivo de peso e alterações no contexto psicossocial já é estabelecida, porém a forma como as diferentes culturas reagem ao ambiente e a relação de causa e efeito entre excesso de peso e estresse psicossocial podem ser diferentes e até bidirecionais (GUNDERSEN et al, 2011; ISASI et al, 2015; ABESO, 2016). O eixo Hipotálamo-Pituitário-Adrenal (HPA) tem sido descrito como desregulado em situações de obesidade abdominal, com direcionamento para alterções nos níveis de cortisol plasmático (RARBER, 1998; EPEL & SEEMAN, 2000). Sabe-se que os glicocorticoides são hormônios de estresse primário, com suas ações mediadas pelos receptores de mineralocorticoide (MR) e de glicocorticoide (GR), com aumento da afinidade pelo segundo, quando em situações de ativação crônica do eixo HPA (COHEN et al, 2012; OAKLEY & CIDLOWSKI, 2013).

O GR, em humanos, é codificado pelo gene NR3C1 e a sua expressão parece ser fortemente influenciada por mecanismos epigenéticos, especificamente a metilação do DNA (a adição de grupos metil às citosinas de ilhas CpG) (OAKLEY & CIDLOWSKI, 2013; STEIGER et al, 2013). A região promotora deste gene tem sido o principal foco dos estudos de metilação de sítios CpG (PALMA-GUDIEL et al, 2015) e a região 1F tem sido

relacionada a situações de estresse psicossocial ao longo da vida (COHEN et al, 2012; YEHUDA et al, 2015; TYRKA et al, 2016).

Diante disto, considerando a bidirecionalidade entre excesso de peso e estresse psicossocial e a multicausalidade de ambos (TORRES; NOWSON, 2007; ISASI et al, 2015; RAZZOLI & BARTOLOMUCCI, 2016), e ainda, partindo da pressuposta associação destes através da hiperatividade do eixo HPA (TURNER & TURNER, 2005; GUNNAR & QUEVEDO, 2007), hipotetizou-se neste estudo que a alteração de peso estivesse relacionada à metilação da região promotora do gene que codifica para o GR (NR3C1).

Na literatura pesquisada até o momento não foram encontrados trabalhos envolvendo o excesso de peso e a metilação do receptor do glicocorticoide, de modo que pouco se sabe se a condição de estresse psicossocial é causa ou consequência da adiposidade e qual seria o impacto ou relação desta síndrome mundial na metilação do NR3C1.

1.1. Objetivos

1.1.1. Objetivo geral

Identificar os indicadores de estresse psicossocial associados à adiposidade abdominal e avaliar a relação entre o excesso de peso e a metilação da região promotora do gene do receptor do glicocorticoide (NR3C1 1F região) em adultos.

1.1.2. Objetivos específicos

- Avaliar a prevalência de excesso de peso e adiposidade abdominal;
- Caracterizar a população quanto ao perfil socioeconômico, de saúde e estilo de vida, segundo localização de moradia rural e urbana;
- Identificar os indicadores de estresse psicossocial associados à adiposidade abdominal em populações rural e urbana;
- Analisar a metilação da região promotora do gene do receptor do glicocorticoide (NR3C1 1F região);
- Avaliar a inter-relação de metilação entre CpGs sítio específicos do segmento avaliado;
- Avaliar a relação entre o excesso de peso e a metilação de NR3C1 1F região.

2. Revisão da Literatura

2.1. Excesso de peso

2.1.1. Aspectos conceituais

Sobrepeso e obesidade representam a manifestação de um fenômeno cultural da atualidade e ao mesmo tempo um reflexo do passado (CARVALHO; MARTINS, 2004). O excesso de peso, bem como suas causas residem em setores que vão para além do setor saúde, como foi muito bem diagnosticado pela diretora-geral da World Health Organization (WHO), configurando-se como um problema de grande preocupação das autoridades públicas de saúde (WHO, 2000; CHAN, 2013).

É importante ressaltar o caráter múltiplo e heterogêneo do excesso de peso. Envolve não apenas fatores biológicos e de causa individual, mas configura-se como uma integração de fatores históricos, econômicos, sociais e culturais que impactam nas escolhas alimentares, nos alimentos disponibilizados e em toda a cadeia de produção de alimentos, colocando em risco não apenas a situação de saúde dos indivíduos como também a sustentabilidade ambiental, econômica e social, tanto no nível local quanto regional (CHOPRA et al, 2002; CARVALHO; MARTINS, 2004; CHAN, 2013).

Segundo a WHO (2000), sobrepeso e obesidade constituem-se num agravo multifatorial que resulta em um desbalanço energético positivo, levando a um acumulo excessivo de gordura. Este acúmulo excessivo de gordura, principalmente na região abdominal é também chamado de adiposidade abdominal e constitui um dos maiores fatores de risco para diversas doenças crônicas como doenças cardiovasculares, diabetes e câncer (CHAN, 2013).

Os fatores ambientais são considerados fundamentais para a compreensão do ganho excessivo de peso nas populações. A determinação da adiposidade consiste no conjunto de fatores relacionados ao modo de vida contemporânea, no qual há uma interposição de fatores de ordem individual (biológicos e comportamentais), fatores relacionados ao modo de comer e viver na atualidade e organização dos sistemas alimentares. Escolhas alimentares, ambientes e comportamento são elementos que geralmente estão presentes conjuntamente, ou seja, ambientes desfavoráveis reforçam comportamentos inadequados que levam a um padrão alimentar não saudável e, consequentemente, favorecem o ganho excessivo de peso (BRASIL, 2014).

Revisões sistemáticas e metanálises tem avaliado a relação de hábitos como assistir televisão, consumo de álcool e horas de sono insuficiente com o aumento na ingestão de alimentos (BEAGAN et al., 2012). Esses comportamentos são todos

conhecidos por afetar as funções cognitivas envolvidas no controle inibitório, podendo representar mecanismos comuns pelos quais a ingestão alimentar é facilitada. A alteração no padrão de consumo alimentar, bem como o aumento da prevalência de doenças crônicas, sobrepeso e obesidade ocorrem em um contexto de mudanças também do sistema alimentar, no qual o modo de se produzir, abastecer, distribuir, comercializar e consumir se encontram majoritariamente orientados para uma lógica de aumento da produtividade e lucro, impactando, por outro lado, negativamente não apenas nos alimentos disponibilizados (ultra processados em sua maioria) como no meio ambiente, na distribuição das riquezas e das terras, nas relações sociais e relações de emprego. Nesse sentido não são os fatores da dieta, como os carboidratos ou a gordura, ou o sedentarismo apenas que devem ser verificados, mas as condições de trabalho, moradia, segurança, rede de abastecimento e globalização que explicam os fatores proximais que, usualmente, são incluídos nos modelos causais das doenças e agravos a saúde (MONTEIRO et al, 2011; CHOPRA et al, 2002; RTVELADZE et al, 2013).

Nesse sentido, a presente contextualização dos determinantes do excesso de peso busca ampliar a discussão fundamentada na hipótese de que trata-se de uma manifestação do corpo e da mente, do biológico, do psicológico e social, ao mesmo tempo individual e coletivo, como muitas das manifestações dos agravos à saúde (SARACENO, 2008). Justifica-se, portanto, a necessidade de empreender investigações interdisciplinares que possam ampliar a compreensão em torno da contextualização do excesso de peso enquanto problema social.

2.1.2. Epidemiologia e fatores associados

O excesso de peso afeta todas as regiões do mundo e vem se configurando como uma epidemia global. Dados atuais da WHO mostram que 38.9% da população mundial com 18 anos ou mais apresenta excesso de peso e, destes, 13.1% são obesos. Observa-se que a prevalência mundial de obesidade duplicou de 1980 até o ano de 2014, acarretando alto índice de mortalidade, totalizando 2,8 milhões de mortes por ano em decorrência de complicações associadas ao excesso de peso. Além disso, 65% da população mundial vive em países no qual o excesso de peso acomete mais indivíduos do que o baixo peso. Essas características epidemiológicas são observadas em todos os países, sendo que, no mundo, 44% da carga de diabetes, 23% da carga de doença isquêmica do coração e entre 7% a 41% de determinados tipos de câncer são atribuídos ao sobrepeso e à obesidade (WHO, 2018).

Em comparação com as outras regiões do mundo, a prevalência de excesso de peso é mais elevada nas Américas (62% para sobrepeso em ambos os sexos e 26% para obesidade em adultos acima de 20 anos de idade) (WHO, 2014). Os países de maiores

prevalências são: Estados Unidos, México e Chile, nos quais o excesso de peso acomete entre 6 e 7 de cada 10 adultos (WHO, 2014; PAHO, 2015). No Brasil, a Pesquisa Nacional de Saúde (PNS) apresentou um cenário semelhante, com 56,9% de adultos com excesso de peso (IBGE, 2015). Em 1974-1975 a prevalência de obesidade era de 10,8%, aumentando para 22,5% e 29,3% nos anos 2002-2003 e 2008-2009, respectivamente, segundo dados da Pesquisa de Orçamento Familiar – POF/2008-2009. A mesma pesquisa indicou, na região sudeste do país, que 50,45% dos indivíduos adultos tinham excesso de peso (IBGE, 2010). Dados ainda não publicados do nosso grupo de pesquisa detectaram uma prevalência de 49,0% de indivíduos com excesso de peso na zona rural do Território Caparaó Capixaba.

Sabe-se que o excesso de peso tem como principais fatores associados: ambiente construído, o ambiente social, comportamento e fatores biológicos de cada indivíduo (BOUCHARD, 2007). O ambiente construído refere-se aos aspectos físicos que são construídos ou modificados pelo homem e geralmente constitui-se de espaço geográfico delimitado em cidades, com áreas rurais e urbanas. Apresenta-se estruturado com elementos que envolvem maior ou menor utilização do solo, disponibilidade de acesso a alimentos, desenho rural ou urbano, diferentes meios de locomoção e transporte, com maior ou menor promoção de atividade física e de lazer. Já o ambiente social se constitui de elementos relacionados às condições de vida como: nível de educação e renda, cultura e crença, sociabilização entre indivíduos, família e vizinhança (BOOTH et al, 2005; FENG et al, 2010). As diversas características dos ambientes construído e social tem sido agrupadas na definição de ambiente obesogênico, o qual favorece o desenvolvimento e manutenção de práticas e comportamentos obesogênicos.

O termo ambiente obesogênico foi proposto, no final da década de 90, como uma espécie de conjunto de influências exercidas sobre indivíduos ou população através do ambiente, das oportunidades e/ou das condições de vida na promoção e desenvolvimento da obesidade (SWINBURN et al, 1999). Neste contexto, acredita-se que o efeito conjunto do estilo de vida, ambiente e comportamento obesogênico seja modulado por características biológicas prevalentes numa determinada população e que a susceptibilidade ao acúmulo excessivo de gordura seja, em parte, determinada pela genética, mas acredita-se também que para a expressão fenotípica seja necessário um ambiente obesogênico (LOOS & BOUCHARD, 2003).

A diferença entre gêneros e a faixa etária constituem, sem dúvida, fatores associados ao aumento do ganho de peso. No que tange os aspectos relacionados ao ambiente social brasileiro, a adiposidade é mais prevalente entre a população de menor renda e de baixa escolaridade (BRASIL, 2014).

Num estudo brasileiro com amostra representativa de adultos no contexto urbano do país, os resultados mostraram associação entre excesso de peso e variáveis do ambiente obesogênico. A privação socioeconômica do bairro e as variáveis do ambiente construído relacionadas à maior mobilidade foram associadas ao excesso de peso na população urbana de uma capital brasileira (MENDES et al, 2013).

Ainda no contexto do ambiente obesogênico, no Brasil destaca-se que os níveis de atividade física e lazer na população adulta são baixos (15%) e apenas 18,2% consomem cinco porções de frutas e hortaliças em cinco ou mais dias por semana, 34% consomem alimentos com elevado teor de gordura e 28% consomem refrigerantes 5 ou mais dias por semana, o que contribui para o aumento da prevalência de excesso de peso e obesidade, que atingem 48% e 14% dos adultos, respectivamente (BRASIL, 2017). O acúmulo excessivo de gordura se expressa em redução da qualidade de vida, maior carga de doenças, dificuldades para o cotidiano de quem é afetado diretamente, para seus familiares e para a sociedade de maneira geral (BRASIL, 2014).

Embora a alimentação adequada e saudável seja um direito humano que deve ser garantido pelo Estado, sabe-se que grande parte da população apresenta hábitos alimentares inadequados, que se relacionam tanto com o desenvolvimento de carências nutricionais como de excesso de peso e doenças crônicas não transmissíveis de alta prevalência atualmente (SEGALL-CORRÊA, 2009). Estes hábitos relacionam-se com o acesso ao alimento e com comportamento alimentar inadequado que, em muitos casos, está associado a estresse e ansiedade que, por sua vez, comprometem a qualidade da alimentação, formando um ciclo (TAYIE & ZIZZA, 2009).

Os fenômenos de transição epidemiológica e demográfica, ocorridos desde o século passado, levaram à modificações nos hábitos de vida, determinando uma maior incidência de doenças crônicas, além de maior exposição à situações estressantes na vida cotidiana. No Brasil, milhares de pessoas migraram da zona rural para a periferia das grandes cidades, em busca de emprego e melhores condições de vida. Entretanto estas mudanças sociais, de hábitos alimentares e de atividade laboral contribuíram para uma maior incidência de sobrepeso e obesidade, bem como na aceleração da vida cotidiana, resultando em maior exposição à situações estressoras ao longo da vida (BRASIL, 2014).

2.2. Estresse e Eixo Hipotálamo-Pituitária-Adrenal

O termo estresse está muito bem estabelecido na literatura ao longo dos anos, e se traduz por todo e qualquer efeito inespecífico de agentes estressores que podem agir sobre o organismo (SELYE, 1950; SINHA & JASTREBOFF, 2013). O estresse pode se manifestar no indivíduo de forma aguda e se estabelecer de forma crônica e, por ambas as formas, se relaciona a alterações no cortisol sérico, mesmo que estas não sejam identificadas

bioquimicamente, uma vez que mecanismos de feedback são ativados como controle e reação aos agentes estressores. A frequente exposição a diversos agentes estressores poderá desencadear uma síndrome de adaptação geral, composta de três fases: reação de alarme, fase de adaptação e fase de exaustão (SELYE, 1950; FONSECA et al, 2009; ISASI et al, 2015). A forma de reação e enfrentamento às adversidades ambientais, econômicas, sociais e de saúde é individulmente diferenciada e, quando as possibilidades de adaptação são ultrapassadas, o estresse psicossocial se torna uma ameaça ao bem estar (TORRES; NOWSON, 2007; FONSECA et al, 2009; GUNDERSEN et al, 2011).

O eixo HPA constitui o principal mecanismo envolvido na fisiologia da resposta ao estresse e é reconhecidamente tão importante para o equilíbrio físico e psíquico que é considerado integrante do sistema Psico-Neuro-Endócrino-Imune (SMITH, 2006; TAUB, 2008; BONAZ & BERNSTEIN, 2013). De forma sucinta, a exposição aos agentes estressores promovem ativação do eixo HPA, primeiramente pela ativação e secreção do hormônio liberador de corticotrofina (CRH) pelo núcleo paraventricular do hipotálamo. Este hormônio estimula a síntese e a secreção do hormônio adrenocorticotrofico (ACTH) pela pituitária anterior, que atua no córtex adrenal, promovendo a síntese e liberação de glicocorticoides (GUNNAR & QUEVEDO, 2007; OAKLEY & CIDLOWSKI, 2013).

Os glicocorticoides são uma família de hormônios esteroides que participam de uma ampla variedade de funções. Nos seres humanos, o cortisol se distribui sistemicamente e executa funções que envolvem os sistemas: imunológico (com papel imunossupressor e anti-inflamatório), digestivo, endócrino, incluindo também a auto regulação do eixo HPA (JOHN et al, 2016). Como uma molécula lipofílica, o cortisol atravessa a membrana celular, por difusão passiva, e liga-se ao receptor citoplasmático mineralocorticóide (MR) e ao receptor de glicocorticoides (GR). MR tem uma maior afinidade para glicocorticóides do que GR, porém, em situações crônicas, a afinidade pelo GR aumenta, representando a etapa de adaptação ao estresse e esta, quando não controlada, pode evoluir para a fase de exaustão (FONSECA et al, 2009; GUNDERSEN et al, 2011; COHEN et al., 2012).

Desta forma, quando o estresse é prolongado, os custos impostos por respostas de estresse frequentes ou prolongadas são descritos como carga alostática. Além disso, os efeitos opostos de MR e GR combinados com a afinidade diferencial dos glicocorticoides mostram que tanto os níveis cronicamente baixos e quanto altos de cortisol estão associados à adaptação não ótima do mecanismo de controle do estresse. As elevações moderadas ou controladas associam-se mais frequentemente à fase de exaustão com provável alteração de saúde física e comportamental (GUNNAR & QUEVEDO, 2007).

Acredita-se que a ativação GR seja a chave para um indivíduo lidar adequadamente com o estresse. Uma vez ligado ao cortisol, o GR pode translocar para o núcleo, desempenhando tanto a transativação de genes regulados pelo eixo HPA quanto a

transrepressão de genes associados a processos pró-inflamatórios, como por exemplo, o NFkB (FARIA, 2006; MILAGRO et al, 2013; ARGENTIERI et al, 2017). Outro ponto a ser considerado neste contexto, é a resistência ao GR, que refere-se a uma diminuição na sensibilidade de células imunes para hormônios glicocorticoides, que normalmente resulta ou intensifica uma resposta inflamatória, aumentando o risco de manifestações agudas, como ocorre na asma e doenças autoimunes, bem como para o aparecimento e progressão de doenças inflamatórias crônicas, como doenças cardiovasculares, obesidade e diabetes mellitus tipo 2 (COLE, 2008).

O estresse psicológico crônico está associado a um maior risco de depressão, doença cardiovascular, diabetes mellitus, doenças autoimunes, infecções respiratórias superiores e dificuldade em cicatrização de feridas (COLE, 2008). Embora estas associações sejam muitas vezes atribuídas a desregulação do eixo HPA, poucos estudos em humanos incluem avaliações de eventos estressantes, resposta eixo HPA e doença nos mesmos indivíduos. A falta de tais estudos é, em parte, atribuível à compreensão ainda parcial dos efeitos do estresse prolongado sobre o eixo HPA em seres humanos e em determinar que mudanças induzidas pelo estresse neste eixo desempenham um papel favorável ao risco de doença. Desta forma, a simples noção de que o estresse crônico atua através dos efeitos diretos de cortisol circulante elevado é cada vez menos provável. O que pode ser mais importante é a forma como tecidos alvo respondem ao cortisol, ao invés dos níveis de hormônio em si (COLE, 2008; COHEN et al, 2012).

2.3. Obesidade e estresse

A obesidade abdominal tem sido associada às perturbações da regulação do eixo HPA. Evidências crescentes sugerem que os efeitos prejudiciais da hipersecreção de glicocorticoide ocorrem pela ativação do eixo HPA em várias patologias humanas, incluindo obesidade (RABER, 1998; EPEL et al, 2000).

Não se classifica a obesidade como um transtorno psiquiátrico, porém, segundo as Diretrizes Brasileiras para a Obesidade, de 2016, da Associação Brasileira para o Estudo da Obesidade e da Síndrome Metabólica (ABESO), existe uma obviedade da relação entre obesidade e transtorno psiquiátrico, dado que o acúmulo excessivo de peso foi, por muito tempo, compreendido como uma manifestação somática de um conflito psicológico subjacente. Essa visão é, ainda hoje, lamentavelmente compartilhada tanto pela população leiga e mesmo por boa parte dos profissionais de saúde. O preconceito com relação à obesidade na infância ainda é frequente, proporcionando um aumento dos sentimentos de inferioridade e do isolamento social entre adultos e crianças obesos. Sintomas de estresse, tais como ansiedade, depressão, nervosismo e o hábito de se alimentar quando problemas emocionais estão presentes são comuns em pacientes com sobrepeso ou obesidade,

sugerindo relação entre estresse, compulsão por comida palatável, transtorno de compulsão alimentar e obesidade. O estresse pode ser uma consequência da obesidade devido a fatores sociais, à discriminação e, alternativamente, a causa da obesidade (ABESO, 2016).

A ativação crônica do eixo HPA e a hipersecreção do cortisol, em humanos, afetam a função do hipocampo e contribuem para o desenvolvimento da obesidade. No cérebro, o hipocampo possui a maior concentração de GR. O estresse crônico induz alterações neuropatológicas, como a atrofia dendrítica nos neurônios do hipocampo, que são paralelos aos déficits cognitivos. A neurotransmissão de aminoácidos excitatórios tem sido implicada na ativação crônica do eixo HPA e estes aminoácidos excitatórios desempenham um papel importante na regulação neuroendócrina. A atrofia dendrítica do hipocampo pode envolver alterações na função transportadora dos aminoácidos excitatórios, e a diminuição desta função também pode contribuir para a ativação crônica do eixo HPA (RABER, 1998).

Descreve-se hiperatividade do eixo HPA, que leva a feedback negativo no núcleo paraventricular, levando à produção de endocanabinóides, que têm efeito no núcleo accumbens levando à busca por alimentos e preparações mais palatáveis e calóricas, as quais tem propriedades de recompensa poderosas no sistema hedônico não homeostático, influenciando o comportamento futuro e levando a maior acúmulo de gordura visceral (EPEL et al, 2000; EHLERT, 2013).

2.4. Epigenética

2.4.1. Aspectos conceituais

O termo epigenética surgiu na metade do século XX, após estudos correlacionando bases genéticas e embriológicas. O prefixo *epi*, do grego, por cima, apresenta uma forma de herança que se sobrepõe à herança genética com base no DNA. Epigenética é definida como o estudo das modificações do DNA e das histonas que são herdáveis e não alteram a sequência de bases do DNA. Dentre as modificações que as histonas podem sofrer, estão: metilação, fosforilação e acetilação. Entretanto, na molécula de DNA ocorre apenas a metilação. O epigenoma, então, é dinâmico e varia de célula para célula dentro de um mesmo organismo multicelular (SZYF, 2007).

As modificações epigenéticas têm início gradual e geralmente são progressivas, mas potencialmente reversíveis e podem ocorrer ao nível do DNA (ex. metilação do DNA) e/ou afetar a estrutura das proteínas da cromatina (histonas) (YOO & JONES, 2006). A metilação do DNA é um importante mecanismo epigenético de regulação da expressão de genes, manutenção, integridade e estabilidade do DNA, modificações cromossômicas e desenvolvimento de mutações (MULERO-NAVARRO & ESTELLER, 2008).

Deste modo, a epigenética traz um conceito bastante inovador no sentido adaptativo, colocando o ambiente como o principal responsável pela condição de expressão dos genes de maneira alterada (DESAI et al., 2015; PROVENÇAL et al, 2015).

2.4.2. Gene do Receptor de Glicocorticoide (GR) (NR3C1)

O GR humano é codificado pelo gene NR3C1, que encontra-se localizado no cromossomo 5, *locus* q31-q32 e apresenta cerca de 140.000 pares de bases (STEIGER et al, 2013). O gene receptor do glicocorticoide contém dezessete exons, sendo oito exons codificantes (numerados de 2 a 9) e nove não codificantes, os quais se localizam no promotor do gene (PALMA-GUDIEL et al, 2015). A região promotora do GR contém múltiplas sequências de dinucleotídeos Citosina—phosphate—Guanina (CpG), denominada "ilha CpG" e esta região não apresenta as seqüências TATA ou CCAAT, as quais fazem parte do complexo de iniciação da transcrição, refletindo a necessidade de expressão constitutiva deste gene. Sete dos primeiros exons não codificantes (D, J, E, B, F, C e H) são agrupados ao longo da mesma ilha CpG. Considera-se que as variantes do exon influenciam a expressão de GR em vários tecidos. A expressão da variante 1D parece ser exclusiva do hipocampo, enquanto as demais são largamente expressas no cérebro, mas também são expressas em vários tipos de tecidos e células sanguíneas (STEIGER et al, 2013). As variantes 1E e 1F são expressas em células do sistema imune, sendo que 1E foi observada tanto em células T CD8+ quanto em monócitos, enquanto a variante 1F foi observada em linfócitos B CD19+ e em células dendríticas do sangue periférico (TURNER & MILLER, 2005).

2.4.3. Processos epigenéticos relacionados ao Gene do Receptor de Glicocorticoide (GR) (NR3C1)

A expressão de genes é fortemente influenciada por mecanismos epigenéticos. Um processo envolvido é a metilação do DNA (a adição de grupos metil às citosinas de CpGs), bastante comum nas regiões reguladoras da maioria dos genes. CpGs metiladas nas regiões promotoras de genes podem reduzir o processo de transcrição e, por sua vez, dificultar ou modular a expressão do gene (STEIGER et al, 2013).

De acordo com a literatura, a expressão do gene do GR (NR3C1) parece ser fortemente influenciada por mecanismos epigenéticos. Em evidência, a privação de cuidados maternos precoce relacionou-se com a hipormetilação do promotor do GR no hipocampo, alteração na expressão do GR e consequente aumento da reatividade ao estresse em animais (WEAVER et al, 2006). Em humanos, abusos sofridos na infância relacionaram-se à hipermetilação sítio específica da região promotora do GR (McGOWAN et al, 2009). Tais processos podem explicar as associações entre as mudanças ambientais

adversas que ocorrem durante o desenvolvimento inicial da vida e os problemas relacionadas à estresse psicossocial, bem como às complicações, patologias e comorbidades associadas na fase adulta (McGOWAN et al, 2009; LABONTE et al, 2012).

A ansiedade e a depressão durante a gestação promove alteração no padrão de metilação do gene receptor do glicocorticoide (NR3C1) humano em recém-nascidos, caracterizando uma modificação epigenética que liga humor materno pré-natal e alteração da homeostase do HPA ocasionando reatividade alterada ao estresse durante a infância (FILIBERTO et al, 2014).

De acordo com Pama-Gudiel et al (2015), o estresse no início da vida constitui fator de risco conhecido para sofrer psicopatologia na idade adulta. A desregulação do eixo HPA foi descrita em indivíduos que experimentaram estresse psicossocial precoce e em pacientes com uma ampla gama de distúrbios psiquiátricos em decorrência do padrão de metilação do gene NR3C1 e a consequente alteração da expressão do receptor do glicocorticoide (GR), o qual constitui elemento-chave envolvido em várias etapas da modulação do eixo HPA. As evidências científicas apontam para a associação das alterações no padrão de metilação do NR3C1 com o estresse de início da vida, bem como psicopatologias e transtornos de estresse pós-traumático. Ainda, segundo estes autores, as principais preocupações das pesquisas da atualidade neste campo tem sido a escolha das ilhas CpG em áreas biologicamente relevantes.

2.4.4. Análise de metilação do DNA

A metodologia tradicional para análise de regiões metiladas envolve o tratamento do DNA com bissulfito como passo inicial, o qual promove a conversão de citosinas não metiladas em uracilas, por desaminação, na presença de NaOH e bissulfito de sódio. Neste tratamento, os resíduos de citosinas não metiladas permanecem intactos. Após conversão por bissulfito, os métodos usados para avaliar quantitativamente a metilação diferem entre os estudos, incluindo: pirosequenciamento, clonagem e sequenciamento de Sanger de regiões de interesse, uso de matrizes de metilação em todo o genoma, espectrometria de massa (MS), entre outros (DASKALAKIS & YEHUDA, 2014).

A utilização de métodos de sequenciação com melhor resolução quantitativa e menor tempo de análise tem sido requerida devido à heterogeneidade e variação na metilação entre células e, neste contexto, o pirosequenciamento tem se destacado. No que se refere à análise de metilação de NR3C1 1F região, os estudos tem utilizado o sequenciamento de Sanger baseado em clones (McGOWAN et al., 2009; YEHUDA et al., 2014b), o pirosequenciamento (OBERLANDER et al., 2008; TYRKA et al., 2012; NA et al., 2014) e espectrometria de massa – MassARRAY (MELAS et al, 2013).

O pirosequenciamento tem apresentado vantagens em relação à clonagem com sequenciamento de Sanger, como o rápido preparo da amostra, automatização do processo com tempo curto de análise e resposta instantânea de sequenciamento e alto rendimento. O alto custo de análise tem se apresentado como desvantagem e, em relação ao MassARRAY, a desvantagem é que a leitura pode se restringir a fragmentos menores de DNA, geralmente menores que 150 pb. Na análise do pirosequenciamento, um dos *primers* de amplificação é biotinilado, isolando-se uma cadeia simples. Posteriormente, a fita simples é purificada e, com o uso de *primers* pirosequenciadores, ocorre a reação em que os nucleotídeos são incorporados sequencialmente, gerando luz por liberação de pirofosfato, o que possibilita a detecção e quantificação (DASKALAKIS & YEHUDA, 2014).

3. Capítulos

3.1. Artigo 1

Tipo: artigo original

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Title: Psychosocial stress and central adiposity: A Brazilian study with users of the public health system

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Abstract

Objective: To assess the association between indicators of psychosocial stress and central adiposity in adult users of the Unified Health System (SUS) from Southeast of Brazil.

Methods: This cross-sectional study was conducted with 384 adults (20 to 59 years old) from the city of Alegre, Southeastern Brazil. The simple random sample represented the population using the public health system of the municipality. The prevalence of obesity was based on the Body Mass Index, and central adiposity (dependent variable) was measured by waist circumference in centimeters. The independent variables were the following indicators of psychosocial stress: food and nutrition insecurity (yes/no), serum cortisol (μg/dL), symptoms suggestive of depression using the Beck Depression Inventory-II ≥ 17 (yes/no), and altered blood pressure ≥ 130/85 mmHg (yes/no). Univariate linear regression was performed between central adiposity and each stress indicator, and later the models were adjusted for socioeconomic, health, and lifestyle variables. All analyses were stratified by rural and urban location.

Results: The prevalence of weight excess was 68.3%, and 71.5% of individuals presented an increased risk for metabolic complications related to central adiposity. Mean waist circumference scores for the rural and urban population were 89.3 ± 12.7 cm and 92.9 ± 14.7 cm, respectively (p = 0.012). Indicators of stress that were associated with central adiposity were: cortisol in the rural population and altered blood pressure in the urban population. This occurred both in the raw analysis and in the models adjusted for confounding factors.

Conclusion: The associations between stress and adiposity were different between rural (cortisol - inverse association) and urban (altered blood pressure) lifestyles, confirming the influence of local and psychosocial subsistence on the modulation of stress and on how individuals react or restrain stressors. Stress reduction strategies can be useful in public health programs designed to prevent or treat obesity.

Keywords: psychosocial stress, central adiposity, obesity, cortisol.

Introduction

Overweight and obesity are defined as abnormal or excessive accumulation of fat that can be detrimental to health.(1) Weight excess affects all regions of the world and is now appearing as a global epidemic. According to the World Health Organization (WHO), 38.9% of the world population aged 18 or more present weight excess and of these, 13.1% are obese.(2) Compared to other WHO regions, the prevalence of weight excess is higher in the Americas (62% for overweight in both sexes, and 26% for obesity in adults over 20

years of age).(3) The prevalence is higher in the United States of America, Mexico, and Chile, where weight excess affects between six and seven out of 10 adults.(3, 4) In Brazil, the National Health Survey (NHS) presented a similar scenario, with 56.9% of overweight adults.(5)

The term stress has already been mentioned in the literature over the years and designates all the non-specific effects of stressors or factors that may act on the body.(6, 7) Stress can be established in the individual in an acute or chronic way, manifested through changes in serum cortisol. Chronic stress can lead to changes in the Hypothalamic-Pituitary-Adrenal (HPA) axis, resulting in altered serum cortisol levels.(8, 9) The frequency of exposure to various stressors can trigger a general adaptation syndrome, composed of three phases: alarm reaction, adaptation phase, and exhaustion phase.(6, 10, 11) The manner in which an individual responds to environmental, economic, social, and health adversities is particularly differentiated, and when the possibilities for adaptation are overcome, psychosocial stress becomes a threat to well-being.(8, 10, 12)

With cultural changes, globalization, and consequent acceleration of the pace of life, there has been an increase in diseases resulting in psychosocial stress and chronic diseases such as obesity, leading concern to public health authorities.(1, 13-19) The relationship between obesity and psychosocial disorders is already well established.(8, 11, 12, 20-22) However, the way different cultures react to the environment and the cause and effect relationship between obesity and stress may be different; these facts along with the issues involved, create a complex concern for authorities. Therefore, this study aimed to evaluate the association between indicators of psychosocial stress and central adiposity in adult users of the Unified Health System (SUS) in a city in the Southeast of Brazil.

Materials and Methods

Study design, sampling, and data collection

This was a cross-sectional study with adults (20 to 59 years, SUS users), which was conducted in Alegre, a city located in the Southeast region of Brazil. It is a municipality with the largest population in the micro-region south of the State of Espirito Santo, Brazil, which has a Human Development Index similar to that of the country (HDI = 0.721 and 0.727, respectively), according to the United Nations Development Program (UNDP).(23)

A population of 10222 individuals, registered in the Primary Care Network of Municipal Health was considered in the study design. For the calculation of the simple random sample, absolute accuracy of 5%, 95% confidence interval, design effect equal to 1 and, in the absence of specific studies in the region, it was estimated that the prevalence of overweight individuals was around 50%. Finally, 10% of losses were added and the sample size calculated was 409 individuals.(24)

The study was approved by the Ethics Committee on Human Research, the Health Sciences Center, Federal University of Espirito Santo, under number 1,574,160 issued on June 03, 2016. The inclusion criteria for participation in the study were the following: not being pregnant, having no cognitive conditions that would interfere with the response to the questionnaires, and declare free consent for participation.

Data collection was performed through an individual interview, with questionnaires that evaluated socioeconomic, health and lifestyle conditions, and food and nutrition insecurity (FNiS), as well as symptoms suggestive of depression. Anthropometric, blood pressure, and blood samples were also collected for cortisol analysis.

The socioeconomic, health, and lifestyle questionnaires were elaborated based on the Individual and Domiciliary Registry Files of SUS, Ministry of Health, Brazil. FNiS was evaluated by the application of the Brazilian Food Insecurity Scale (BFIS)(25, 26), a validated instrument comprising 14 questions, aimed at families from the same household with and without members under 18 years of age; concerns evaluated were: lack of food at home, and having some member of the family spending a whole day without eating in the last three months, among others. The degree of severity of FNiS (mild, moderate, and severe) was grouped in this study.

Symptoms suggestive of depression were investigated using the Beck Depression Inventory-II (BDI-II), and total scores were categorized according to the literature: normal, mild, moderate, and severe.(27-29) For the purpose of this study, the following regrouping was used: normal or mild mood disorder (BDI-II <17), and symptoms suggestive of depression (BDI-II ≥ 17).

The anthropometric evaluation was performed by qualified professionals in the morning, after participants had fasted for a minimum of eight hours, following the technical standards of the Food and Nutritional Surveillance System (SISVAN).(30) Stature was evaluated using Alturexata® stadiometer, with a maximum capacity of 2.10 m and accuracy of 0.5 cm. The weight was measured on a Tanita® bipolar bioimpedance balance, with a BC601® branded body fat monitor (with 100 g division and maximum capacity of 150 kg). The Body Mass Index (BMI) was calculated and classified according to WHO reference for adults(1), in which low weight individuals present BMI values < 18.5 kg/m^2, eutrophic range from 18.5 to 24.9 kg/m^2, overweight from 25.0 to 29.9 kg/m^2, and obese individuals BMI being ≥ 30.0 kg/m^2. Waist circumference (WC) was measured using an inextensible anthropometric tape TBW® (1.5 m and 0.1 cm accuracy), with reference to the midpoint between the last rib and the iliac crest. (31, 32) This study considered that increased risk of metabolic complications for men was associated with abdominal obesity with WC values ≥ 94 cm; and very high risk with WC ≥ 102. For women, an increased risk of metabolic

complications was considered at/with WC values ≥ 80 cm, and very high risk, with WC values ≥ 88 cm.(1, 32)

Blood pressure was measured using an aneroid sphygmomanometer with a G-TECH® Premium model. Both the measurement technique and the blood pressure classification were based on the VI Brazilian Guidelines for Hypertension from the Brazilian Society of Cardiology (SBC) for individuals 18 years and older(33), considering altered blood pressure (AP) when values are ≥ 130/85 mmHg.

Blood collection for cortisol analysis was performed in the morning, between 7:00 and 9:00 a.m. The samples were collected in a separator gel tube, kept at room temperature until the clot was retracted, then centrifuged at 2500 rpm for 10 min and refrigerated at 2 ° C to 8 ° C until analysis. The concentration of cortisol was quantified by the chemiluminescence method and the reference values for the morning dose were 6.7 to 22.6 µg / dL.(34)

Data analysis

Data were tabulated and submitted for consistency analysis. Individuals who presented inconsistency of anthropometric data and those who reported the use of corticosteroid medications were excluded from the analysis.

For the descriptive analysis, the variables were presented as medians and interquartile ranges, or means and standard deviations (non-parametric or parametric data, respectively, according to Kolmogorov-Smirnov normality test), besides frequencies and proportions. The Mann-Whitney U test or Student's t-test and the chi-square test were used to verify the differences related to the rural and urban locations, considering a level of significance of 5%. In the chi-square test, for the variables that had three or more categories and that presented significant differences, 2 x 2 ratio analysis was performed later, using the Bonferroni correction, which changes the level of significance (p), to avoid type I errors derived from multiple comparisons. After this procedure, the corrected significance level was $p<0.016$.(35)

Univariate linear regression models were used to evaluate the association between stress indicators and central adiposity. All statistical analyses were performed using SPSS® software, version 15.0 for Windows (IBM, Munich, Germany).

The univariate linear regression analysis consisted of simple models (crude analysis) hierarchically adjusted in three different models: Model 1, analysis adjusted by socioeconomic variables; Model 2, adjusted by socioeconomic and health variables; and Model 3, adjusted by socioeconomic, health, and lifestyle variables.

Outcome variable: Central adiposity

In this study, the WC in centimeters was chosen as an outcome variable to predict central adiposity from the explanatory variables; it was included in the models as a continuous variable.

Independent variables

The independent variables selected for the univariate analyses were: FNiS (yes/no), serum cortisol (μg/dL), symptoms suggestive of depression with BDI-II ≥ 17 (yes/no), and AP ≥ 130/85 mmHg (yes/no), which are directly or indirectly related to psychosocial stress. All these indicators were measured by methods and/or validated instruments with good reproducibility in previous studies.

Potential confounding variables

Socioeconomic variables (age, sex, schooling, and income classification), health (stress report, one or more comorbidities reported, and self-rated health), and lifestyle (current smoking, current alcohol consumption, and weekly physical or leisure activity) were chosen to adjust the univariate linear regression models based on the knowledge collected from the preexisting literature regarding the relation between psychosocial stress and overweight.(7, 8, 12, 14, 36)

Among the socioeconomic variables, it is highlighted that schooling was evaluated in years of study and income was classified according to the Center for Social Policies of the Getúlio Vargas Foundation, which considers low-income individuals with a per capita income of less than US$ 5 per day.(37) In the case of the variable comorbidities, the response options were diabetes, hypertension, cardiac palpitation, cardiovascular disease, and metabolic syndrome, conditions known to be associated with obesity and physical stress.(8) The practice of physical activity was added to leisure, and, as a reference, it was considered that once a week was the minimum frequency to "relief/escape" from the stressful routine, but not to be seen as the ideal.

Modeling

Beyond the WC (outcome variable), the variables age and serum cortisol entered the models as continuous variables, and all others as categorical and dichotomic, as shown in Table 1.

Table 1. Treatment of variables for modeling

Variables	Dichotomization
Dependent variable	
WC (cm)	continuous variable
Independent variables / Stress indicators	
FNiS	yes* / no
Serum cortisol (µg/dL)	continuous variable
BDI-II ≥ 17	yes* / no
AP ≥ 130/85 mmHg	yes* / no
Adjustment variables	
Socioeconomic conditions	
Age	continuous variable
Sex	female* / male
Years of study	< 9* / ≥ 9
Income classification (< $5.00/day)	low income* / non-low income
Health conditions	
Stress report	yes* / no
1 or + comorbidities reported	yes* / no
Self-rated health	regular or bad * / good or very good
Lifestyle	
Current smoking	yes* / no
Current alcohol consumption	yes* / no
Physical or leisure activities (≤ 1 time/week)	yes* / no

* indicates the reference category. WC: waist circumference; FNiS: Food and Nutritional Insecurity; BDI-II: Beck Depression Inventory-II; AP: Amended Pressure.

The univariate analyses for the rural and urban populations were performed separately, to evaluate the differences in the behavior of the predictor variables regarding

the stratification of the models by location. All variables used in the models met the assumption of collinearity. The assumptions of normality, linearity, homoscedasticity, and independence of residues were met in all models that had p <0.05 in the F test. Results are presented as non-standard regression coefficients (β), and their respective 95% confidence intervals (CI) and p-values related to the explanatory variable. All analyses were performed using the complete sample, and the significance level was 5% (p <0.05).

Results

A total of 384 individuals participated in the study, 75 men and 309 women, corresponding to 19.5% and 80.5%, respectively. Among the participants, 133 were rural residents, and 251 lived in urban areas. The median age was 42.5 years (Interquartile Ranges - IR=18.0), and there was no significant difference between the urban and rural populations (p=0.82) or between men and women (p=0.23).

The prevalence of overweight was 33.1%, and that of obesity was 35.2%. Regarding the risk of metabolic complications related to central adiposity (WC, in cm), 51.6% of the sample presented increased risk and the risk of 19.9% substantially increased. The median BMI and WC mean scores were higher in the urban than in the rural populations. The other characteristics related to health, socioeconomic conditions, and lifestyle of the population are presented in Table 2.

Tables 3 and 4 present the results of the association between indicators of psychosocial stress and central adiposity in the rural and urban populations, respectively. In the rural population, serum cortisol was inversely associated with central adiposity in all models. AP was associated with adiposity only in the crude model, losing significance in the adjusted models (Table 3). The evaluation done in the urban population showed that only AP was associated with central adiposity and this occurred in all models (Table 4). The other stress indicators evaluated – FNiS and BDI-II> 17 – had no association with central adiposity in the study population (Tables 3 and 4).

Table 2. Characteristics of the population according to rural and urban locations

Characteristics	All	Rural	Urban	p*
Health profile				
Nutritional Status - BMI				
Low weight	1.6 (6)	1.5 (2)	1.6 (4)	0.116**
Eutrophy	30.2 (116)	33.1 (44)	28.7 (72)	
Overweight	33.1 (127)	36.8 (49)	31.1 (78)	
Obesity	35.2 (135)	28.6 (38)	38.6 (97)	
BMI (kg/m²) – median (IR)	27.5 (7.8)	26.8 (7.3)	28.4 (8.1)	**0.027**
Risk metabolic complications - WC				
Low	28.5 (109)	31.8 (42)	26.8 (67)	0.084**
Increased	19.9 (76)	24.2 (32)	17.6 (44)	
Substantially increased	51.6 (197)	43.9 (58)	55.6 (139)	
WC (cm) - mean (±SD)	91.7 (±14.1)	89.3 (±12.7)	92.9 (±14.7)	**0.012**
Classification of cortisol - % (n)				
Low	10,0 (37)	15,0 (19)	7,4 (18)	0,042**
Normal	87,8 (324)	81,9 (104)	90,9 (220)	
High	2,2 (8)	3,1 (4)	1,7 (4)	
Serum cortisol (µg/dL) - median (IR)	12.1 (6.1)	10.8 (5.5)	12.9 (6.2)	**0.006**
Depression - % (n)				
BDI-II < 17	76.4 (272)	77.5 (93)	75.8 (179)	0.728
BDI-II ≥ 17	23.6 (84)	22.5 (27)	24.2 (57)	
AP ≥ 130/85 mmHg - % (n)				
No	79.8 (296)	87.5 (112)	75.7 (184)	**0.007**
Yes	20.2 (75)	12.5 (16)	24.3 (59)	
Stress report - % (n)				
No	23.2 (89)	16.7 (22)	26.6 (67)	**0.029**
Yes	76.8 (295)	83.3 (110)	73.4 (185)	
Report of comorbidities - % (n)				
No report	65.7 (251)	70.2 (92)	63.3 (159)	0.179
One or more comorbidity	34.3 (131)	29.8 (39)	36.7 (92)	
Use of continuous medication - % (n)				
No	45.1 (173)	46.6 (62)	44.2 (111)	0.630
Yes	54.9 (211)	53.4 (71)	55.8 (140)	
Self-rated health - % (n)				

Good or very good	46.9 (180)	42.1 (56)	49.4 (124)	0.173
Regular or poor	53.1 (204)	57.9 (77)	50.6 (127)	
Socioeconomic profile				
BFIS Classification - % (n)				
FNS	58.2 (223)	60.6 (80)	57.0 (143)	0.493
FNiS	41.8 (160)	39.4 (52)	43.0 (108)	
Age (years) - median (IR)	42,5 (18,0)	42,0 (18,0)	43,0 (18,0)	0,820
Skin color - % (n)				
White	54.7 (205)	57.3 (75)	53.3 (130)	0.461
Non-white	45.3 (170)	42.7 (56)	46.7 (114)	
Marital status - % (n)				
Single	24.7 (95)	25.6 (34)	24.3 (61)	0.819
Not single	75.3 (289)	74.4 (99)	75.7 (190)	
Years of study - % (n)				
≥ 9 years	54.0 (204)	40.9 (54)	61.0 (150)	**<0.001**
< 9 years	46.0 (174)	59.1 (78)	39.0 (96)	
Income classification - % (n)				
Non-low income (≥ $5.00/ day)	58.5 (224)	45.1 (60)	65.6 (164)	**<0.001**
Low income (< $5.00/ day)	41.5 (159)	54.9 (73)	34.4 (86)	
Lifestyle				
Physical or leisure activities - % (n)				
≥ 1 time/ week	86.1 (322)	84.9 (107)	86.7 (215)	0.640
Non or ≤ 1 time/ week	13.9 (52)	15.1 (19)	13.3 (33)	
Current smoking - % (n)				
No	91.9 (352)	94.7 (125)	90.4 (227)	0.146
Yes	8.1 (31)	5.3 (7)	9.6 (24)	
Current alcohol consumption - % (n)				
No	68.9 (264)	78.8 (104)	63.7 (160)	**0.003**
Yes	31.1 (119)	21.2 (28)	36.3 (91)	

BMI: Body Mass Index; WC: Waist Circumference; AP: Amended Pressure; BFIS: Brazilian Food Insecurity Scale; FNS: Food and Nutrition Security; FNiS: Food and Nutrition Insecurity; BDI-II: Beck Depression Inventory-II. Quantitative variables presented in medians and interquartile ranges (IR), or means and standard deviations (±SD) according to normality (Kolmogorov-Smirnov test); categorical variables presented in relative (%) and absolute (n) frequencies. * p-value for the tests: Mann-Whitney U or Student t and chi-square, at 5% significance and, when three or more categories, corrected by Bonferroni. ** 5% significance, adjusted by Bonferroni (p <0.016).

Table 3. Association between indicators of stress and central adiposity in the rural population

Stress indicators	**Univariate linear regression**			**Univariate linear regression models adjusted for confounding variables**								
	Gross analysis			***Model 1***			***Model 2***			***Model 3***		
	β	**95%CI**	**p**	**β**	**95%CI**	**p**	**β**	**95%CI**	**p**	**β**	**95%CI**	**p**
FNiS	-0.13	-4.62; 4.37	0.956	-0.42	-5.21; 4.38	0.864	-1.28	-6.21; 3.65	0.608	-1.11	-6.34; 4.12	0.675
Serum cortisol	-0.61	-1.06; -0.15	**0.010**	-0.51	-0.98; -0.04	**0.033**	-0.58	-1.05; -0.11	**0.015**	-0.60	-1.09; -0.11	**0.018**
BDI-II ≥ 17	4.62	-0.71; 9.96	0.089	5.16	-0.06; 10.38	0.053	4.31	-1.45; 10.06	0.141	4.95	-1.57; 11.47	0.135
AP	6.77	0.22; 13.33	**0.043**	2.68	-4.35; 9.71	0.452	-1.72	-9.80; 6.36	0.674	-4.71	-13.64; 4.22	0.298

Complex sample. Univariate linear regression. Significance level of 5% ($p < 0.05$). Dependent variable: Waist circumference (WC) in cm; Stress indicators (independent variables): Serum cortisol (in µg / dL); Food and Nutrition Insecurity – FNiS (yes); Beck Depression Inventory-II – BDI-II ≥ 17 (yes); Amended Pressure – AP ≥ 130/85 mmHg (yes). Gross analysis and univariate linear regression models hierarchically adjusted for confounding factors: *Model 1* - gross analysis adjusted for socioeconomic variables (sex, age in years, schooling, and income status); *Model 2* – gross analysis, adjusted for socioeconomic variables (sex, age in years, schooling, and income status), and health variables (stress report, reporting one or more comorbidities, and self-rated health); *Model 3* – gross analysis, adjusted for socioeconomic variables (sex, age, schooling, and income status), health variables (stress report, reporting of one or more comorbidities, and self-rated health), and lifestyle variables (current smoker, current alcohol consumption, and practice of physical activity and/or weekly leisure). β: beta coefficient; 95% CI: 95% confidence interval; p: p-value. The variables used met the assumption of collinearity. The assumptions of normality, linearity, homoscedasticity, and independence of residues were met in all models that had $p < 0.05$ by F test.

Table 4. Association between indicators of stress and central adiposity in the urban population

Stress indicators	Univariate linear regression			Univariate linear regression models adjusted for confounding variables								
	Gross analysis			*Model 1*			*Model 2*			*Model 3*		
	β	**95%CI**	**p**	**β**	**95%CI**	**p**	**β**	**95%CI**	**p**	**β**	**95%CI**	**p**
FNiS	-0.66	-4.37; 3.04	0.725	-0.62	-4.59; 3.35	0.758	-1.22	-5.31; 2.87	0.558	-0.65	-4.81; 3.51	0.759
Serum cortisol	-0.21	-0.62; 0.19	0.294	-0.17	-0.59; 0.24	0.409	-0.19	-0.61; 0.22	0.359	-0.27	-0.69; 0.15	0.211
BDI-II ≥ 17	1.80	-2.66; 6.25	0.427	1.59	-3.08; 6.26	0.503	0.82	-4.02; 5.65	0.740	1.64	-3.27; 6.55	0.512
AP	9.66	5.52; 13.80	**<0.001**	8.41	-4.05; 12.76	**<0.001**	7.07	2.59; 11.55	**0.002**	6.66	2.14; 11.18	**0.004**

Complex sample. Univariate linear regression. Significance level of 5% (p <0.05). Dependent variable: Waist circumference (WC) in cm; Stress indicators (independent variables): Serum cortisol (in µg / dL); Food and Nutrition Insecurity – FNiS (yes); Beck Depression Inventory-II – BDI-II ≥ 17 (yes); Amended Pressure – AP ≥ 130/85 mmHg (yes). Gross analysis and univariate linear regression models hierarchically adjusted for confounding factors: *Model 1* - gross analysis adjusted for socioeconomic variables (sex, age in years, schooling, and income status); *Model 2* – gross analysis, adjusted for socioeconomic variables (sex, age in years, schooling, and income status), and health variables (stress report, reporting one or more comorbidities, and self-rated health); *Model 3* – gross analysis, adjusted for socioeconomic variables (sex, age, schooling, and income status), health variables (stress report, reporting one or more comorbidities, and self-rated health), and lifestyle variables (current smoker, current alcohol consumption, and practice of physical activity and/or weekly leisure). β: beta coefficient; 95% CI: 95% confidence interval; p: p-value. The variables used met the assumption of collinearity. The assumptions of normality, linearity, homoscedasticity, and independence of residues were met in all models that had p <0.05 by F test.

Discussion

This study evaluated the association between indicators of psychosocial stress and central adiposity in adults of a city in Southeast Brazil, and users of the Unified Health System (SUS). The health conditions of the Brazilian population, as well as other nations, transcend the concept of absence of diseases resulting from innumerable social, economic, environmental, and cultural factors. The main health problems of people living in poverty include increased exposure to environmental risk factors, diseases (especially non-communicable diseases), poor nutritional status, difficult access to health services and medicines, as well as other economic, psychosocial, cultural, or health factors, which act as stressing agents, affecting well-being and social interaction.(38)

This study was performed on a population that frequently uses the public health system, which presented socioeconomic (mainly in the rural area) and health fragility, including self-perception reports (53.1% reported poor or regular health), which may represent an impairment of the individual and collective state of well-being, expressing as continuous stress. This, in turn, tends to feed the same cycle of psychosocial instability and lack of health and well-being in a chronic way.

The prevalence of weight excess (BMI ≥ 25.0 kg / m2) found in the present study (68.3%) was equivalent to the current US data and surpassed the weight excess data of the world population (52.0%)(2), of the Brazilian adult population (56.9%)(5), the population of the state of Espirito Santo (52.4%)(39), and of its capital Vitoria (49.7%).(40)

Data from the Family Expense Research - POF 2008-2009(39) showed a lower prevalence of overweight and obesity in rural Brazil (38.8% and 8.8%, respectively), whereas in the present study the prevalence of overweight and obesity were high and statistically similar, both in rural and urban areas (Table 2).

Added to this context, the results were worse when the WC assessment was expanded, with a prevalence of 71.5% at risk of metabolic complications associated with central adiposity, with 51.6% of the individuals at high risk. This proportion exceeded the national prevalence stated in the National Health Survey – NHS(5), which was 37.0%, using the same cut points.(1, 32)

Based on the literature, the main socioeconomic, health, and lifestyle indicators related to excess weight were considered in the present study (Table 2)(5, 9, 14, 26, 39, 41), which have also been referred to in studies as psychosocial stressors.(7-9, 11, 42)

Socioeconomic variables such as schooling and income are indicators of poverty and, together with the FNiS indicator, may represent a direct relation of hunger, and scarcity, with deficiency and malnutrition states.(25, 43, 44) The socioeconomic profile traced in this study pointed to a high prevalence of FNiS (41.8%) in the general population, characterized by low schooling and income, especially in the rural population (Table 2). The nutritional assessment pointed to 30.2% having eutrophy, and only 1.6% having low weight, distancing the population from the limit of 5% that would characterize the presence of current malnutrition.(5) On the other hand, it is known that poverty indicators are also related to excess caloric intake(45) and, consequently, to adiposity. Under these conditions, adiposity may even mask situations of nutritional deficiencies, which is often the case in less developed regions with populations of low schooling and income.(13, 14, 45)

FNiS was one of the variables chosen to evaluate the association between psychosocial stress and adiposity, since this indicator represents scarcity and is related to fear of hunger, insecurity, fragility, and individual and familial instability, which could trigger

the activation of the HPA axis, with chronic stress setting. However, FNiS had no association with central adiposity (Tables 3 and 4).

In the context of analysis of health variables, 15% of the individuals living in rural areas had low levels of serum cortisol. Although the prevalence of hypocortisolemia was statistically similar between rural and urban areas, the median serum cortisol was significantly lower in the rural population than in the urban population ($p = 0.006$), according to Table 2. In addition, in evaluating the association of stress indicators with central adiposity, serum cortisol was inversely associated with central adiposity in the rural population in all univariate analyses (gross analysis and confounding-adjusted models, as in Table 3). In the urban population, serum cortisol was not associated with adiposity (Table 4). The association found in the rural population was contrary to what has been observed in some studies on the relationship between stress and obesity, which have justified the weight gain, due to the increase in intake of palatable and high caloric foods, as a compensatory mechanism resulting in hypercortisolemia of chronic HPA axis activation(7, 8, 20, 46); or weight gain due to the deregulation of satiety mechanisms, through alterations in the leptin system, and also resulting from high levels of cortisol.(7, 12)

On the other hand, the notion that chronic stress acts simply by the direct effect of elevated serum cortisol is becoming less likely relevant in describing how the target tissues respond to cortisol, rather than the levels of the hormone itself.(9) Stressors trigger an HPA axis response by regulating corticotrophin-releasing factor and cortisol feedback and, over time, chronic overexposure to stressors may result in a blunted stress response with decreased levels of cortisol and other associated neurobiological dysfunctions.(47-49) In the absence of supportive care, stressors experienced during life, especially in early life and developmentally sensitive periods, such as pregnancy, childhood, and adolescence, may leave impressions on the neural substrate of emotional and cognitive processes, which may result in a blunted response to cortisol and consequent resilience of the individual to psychosocial stressors.(49) Regarding obesity, hyperactivation of the HPA axis has been observed in obese individuals, but also in individuals with paradoxically normal or low plasma cortisol levels. In a study by research colleagues(50), obesity was associated with relative insensitivity to glucocorticoid feedback. The authors suggested that this condition is characterized by a decreased response of the mineralocorticoid receptor to circulating cortisol.

However, the associations between cortisol and obesity appear to be more complex than anticipated(51). Although it promotes changes in the compensatory mechanism of food intake(7, 8, 20, 46), in the leptin system and satiety control mechanism(7, 8), cortisol is clearly not the only peripheral trigger of adverse effects, which may explain the controversies in this area. It seems likely that the pattern in which cortisol is secreted in

front of the stressors is as important as total cortisol secretion. Thus, there is relevance in exploring the social, psychological conditions, health, and lifestyle of a population to identify the main psychosocial stressors and how individuals react and resist to stress.

Another indicator of psychosocial stress used in the present study was depression using BDI-II scores ≥ 17. It is known that early life stress is a risk factor for psychopathology in adulthood, to the extent that the HPA axis was described as deregulated in individuals who experienced early psychosocial stress as well as in those with a wide range of psychiatric disorders.(52) In studies done by colleagues(53) related to chronic stress, depression, and location of urban neighborhoods, the daily stress of living in neighborhoods where residential mobility and material deprivation prevailed was associated with depression. Another study showing the association between urban versus rural environments and depression in the Scottish population found a higher prevalence of psychotropic medication prescription for anxiety, depression, and psychosis in the urban population.(54)

In the present study, a prevalence of depression was found in 23.6% of the population assisted by SUS, with a statistical similarity between rural and urban areas (Table 2). This indicator was not associated with adiposity in any of the models, according to Tables 3 and 4.

In the urban population, the evaluation of health variables pointed to higher levels within the following indicators: median BMI and cortisol, mean WC, and prevalence of high blood pressure (24.3% of individuals with AP), corroborating data from the literature related to hypertension and obesity in urban areas.(55, 56) Lifestyle assessment also showed higher alcohol consumption in the urban population (Table 2). Finally, AP was the only indicator of stress associated with adiposity in the urban population.

It is known that among the main risk factors for elevated blood pressure in adults are obesity, high sodium intake, smoking, alcohol consumption, psychological factors, and certain personality traits such as stress and anxiety.(57-59) Most studies report obesity as a predictor of arterial hypertension(58-63); however, in this study, it was hypothesized that AP, as a psychosocial stress indicator, could predict the increase of WC, which occurred in all models tested (Table 4). It is recalled here that, in the rural population (Table 3), AP was also associated with central adiposity, but this association occurred only in the gross analysis since the adjusted models for confounding variables lost their significance.

Finally, as part of the initial hypothesis, significant differences were observed between home locations. Socioeconomic conditions were challenging in the rural area regarding income and schooling, despite the statistical similarity in the prevalence of FNiS between rural and urban areas. In addition, the report of stress as a personality trait was higher in the population living in the rural region which, on the other hand, presented the

lowest median for serum cortisol. As mentioned before, the urban population exceeded the rural population regarding the prevalence of AP. Both the literature review data(53, 54, 64, 65) and the findings of this study reinforce the influence of socioeconomic and livelihood conditions on the different mechanisms of adaptation of the organism to stressors, and on the different forms of coping and developing resilience.

Conclusions

The stress indicators that were associated with central adiposity were serum cortisol (with an inverse association) in the rural environment and altered blood pressure in the urban area. The study considered several variables known to be associated with stress and weight gain and the models presented robustness in the explanation of the results found.

The observed differences in adiposity prediction regarding housing location reinforce the influence of the local and psychosocial environments on the modulation of stress and on how individuals react to or restrain stressors. Stress reduction strategies can be useful in public health programs designed to prevent or treat obesity.

The study suggests the need for a better evaluation of the use of cortisol as a marker, since both high and low cortisol may explain variations in health status. Both levels relate to different forms of coping or resilience to psychosocial stress. Additionally, owing to the possible bidirectionality of the association between stress and adiposity, new paths should be drawn in the context of a thorough investigation of the mechanisms that explain psychosocial stress, with hypocortisolemia as a biomarker and adiposity as a consequence of current lifestyle.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: AMAS FVF. Performed the experiments: FVF WMB LAAS MJOG JAP ARB CLC JKA HP MMO ABA EASF. Analyzed the data: FVF AMAS WMB EBB IDL DRO. Contributed reagents/materials/analysis tools: FVF WMB LAAS MJOG JAP ARB CLC JKA HP ABA DRO EBB IDL AMAS. Wrote the paper: FVF AMAS. Research Project Coordinator: AMAS.

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3.2. Artigo 2

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Title: Overweight is associated with hypomethylation of the glucocorticoid receptor gene (NR3C1 1F region) promoter region

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Abstract

The hyperactivation of the hypothalamic pituatary adrenal (HPA) axis is related to psychosocial stress and also to excessive accumulation of weight. The aim of this study was to investigate the association between overweight and methylation of the glucocorticoid receptor gene (NR3C1) promoter 1F region in adults. This cross-sectional study evaluated 282 adults with regard to Body Mass Index (BMI), being categorized into two groups: non-overweight and overweight (94 vs. 188 individuals). The methylation analysis of the NR3C1 1F region by pyrosequencing showed that the overweight group had lower methylation

levels than the non-overweight group both for total and Cytosine-phosphate-Guanine (CpG) site specific methylation ($p < 0.05$ and $p_{corrected} \leq 0.037$). By factor analysis, two bins of CpGs were extracted and intercorrelated, and hypomethylation in these bins and in the total segment was explained by overweight when controlled by covariates. As this is one of the first studies examining weight accumulation and methylation in the 1F promoter region of glucocorticoid receptor (GR), we suggest that future research is needed to further elucidate the epigenetic mechanisms involved in this complex and multifactorial pathway involving psychosocial stress and excessive weight gain.

Keywords: overweight, DNA methylation, NR3C1, epigenetic, glucocorticoid receptor

Introduction

The excessive accumulation of weight affects all regions of the world, manifesting itself as a multiple and heterogeneous epidemic.[1, 2] Excessive weight accumulation occurs due to biological and environmental factors, ranging from complex genetic characteristics to the integration of historical, economic, social and cultural factors that impact and endanger the health of individuals.[2]

Abdominal obesity has been associated with disorders in the regulation of the hypothalamic pituitary adrenal axis (HPA axis), with its orientation towards paradoxically altered or normal levels of plasma cortisol.[3, 4] Glucocorticoids are primary stress hormones and their actions are mediated by the affinity mineralocorticoid receptor in physiological and acute elevation states, as well as by the glucocorticoid receptor (GR) through increasing affinity in situations of chronic HPA axis activation.[5, 6]

GR is a member of the nuclear receptor superfamily of binder-dependent transcription factors. When occupied by glucocorticoids, it is capable of inducing or repressing the transcription of many genes by direct binding to the DNA response elements and/or by physically associating with other transcription factors.[6] In humans, GR, is encoded by the NR3C1 gene, which is located on chromosome 5, locus q31-q32 and has about 140,000 base pairs.[7, 8] This gene contains seventeen exons, eight coding exons (numbered 2 to 9) and nine non-coding exons, which are located in the gene promoter.[9] The GR promoter region contains multiple Cytosine-phosphate-Guanine (CpG) dinucleotide sequences, and does not present TATA or CCAAT sequences, part of the transcription initiation complex, reflecting the need for constitutive expression of this gene.[10] Seven of the first non-coding exons span 3 kb of the proximal promoter region of the gene, grouping within the same CpG island, in which epigenetic surveys have been concentrated.[9]

According to the literature, GR expression appears to be strongly influenced by epigenetic mechanisms, specifically DNA methylation (the addition of methyl groups to CpG island cytosines), which may lead to alterations in chromatin architecture and inhibit binding of transcription factors and often results in altered gene expression.[7, 11, 12, 13] Consistent with this, genes such as NR3C1, that are highly expressed, typically have low levels of promoter methylation.[14] The promoter region of the gene has been the main focus of methylation studies of CpG sites[9] and the 1F region has been related to situations of adversity in early life [14], including post-traumatic stress disorder[15], childhood maltreatment and abuse[12, 16], maternal depression and anxiety[17, 18], and other psychiatric disorders[14], in addition to other chronic situations involving psychosocial stress[5] and poor health.[19]

In view of this, and considering the bidirectional relationship between stress and obesity[20, 21, 22], we hypothesized that weight change may be related to the methylation of the NR3C1 1F region. Starting from the presupposed relationship between HPA axis hyperactivation, due to chronic stress[23, 24], and its correlation with body weight change [25, 26, 27], our objective was to evaluate the association between overweight and methylation of the NR3C1 promoter 1F region in adults.

Results

We observed an overweight prevalence of 66.7% (BMI ≥ 25.0 kg / m2), and a comparison of characteristics between the non-overweight and overweight groups showed differences in age and biochemical profile with regards to glycemia, total cholesterol level and fractions, and triglycerides, as described in Table 1.

CpG site specific methylation levels are graphically represented in Figure 1A and the comparison between non-overweight (n = 94) and overweight (n = 188) groups can be seen in Figure 1B. The median values observed were low, but were significantly lower in the overweight group. This group had a significantly lower percentage of methylation than the non-overweight group in the following specific CpG sites: 41, 42, 44 and 45, as shown in Figure 1B.

By means of factorial analysis it was possible to analyze the interaction between the CpGs and the model generated by the extraction of two main components, which we named bin 1: CpG40-41-43 and bin 2: CpG42-44-45, with 61.8% of total variation in the segment analyzed, as seen in Table 2.

Total methylation, given by the sum of methylation percentages of the CpG 40 to 45 segment and the methylation of bins 1 and 2, as well as the comparison of methylation between groups, respectively, are represented in Figure 2 (A and B). We observed that the

overweight group had significantly lower percentages of methylation independent of the analyzed segment (Figure 2B, $p < 0.05$).

Regarding the prevalence of methylation in the NR3C1 1F region, we found a proportion of 26.6% individuals with methylation in the total segment (CpG 40 to 45). The prevalence of methylation in bin 1 (CpG 40-41-43) and bin 2 (CpG 42-44-45) were 26.2% and 3.9%, respectively, as shown in Figure 2C.

In the Spearman correlation analysis, we found that overweight correlated with hypomethylation in CpGs 42 ($r = -0.148$, $p = 0.013$), 44 ($r = -0.142$, $p = 0.017$), and 45 ($r = -0.153$, $p = 0.010$), as well as within bin 2 ($r = -0.137$, $p = 0.021$).

The results of Poisson regression with robust variance showed that overweight reduces the prevalence of total methylation by about 40%, and of methylation in bin 1 (CpG40-41-43) by about 38%, when controlled by the effect of alcoholic beverage use, which was also significant in reducing the prevalence of methylation. The prevalence ratio of methylation in bin 2 (CpG42-44-45) in overweight individuals was 0.23, that is, overweight reduces the prevalence of methylation in this CpG bin by approximately 77%, when controlled by alteration of lipid profile, which was also associated with the lower prevalence ratio. All models were controlled by the covariables of confusion and were statistically significant, but only the first two models showed strong adherence to the Hosmer & Lemeshow adjustment, as shown in Table 2.

Table 1. Characteristics of the study population according to overweight

Characteristics	Overweight (BMI ≥ 25 kg/m²)		
	No	**Yes**	**p**
Sex - % (n)			
Male	35.0 (21)	65.0 (39)	0.758
female	32.9 (73)	67.1 (149)	
Age (years) - median (IR)	39.5 (19.0)	44.0 (17.0)	0.017*
Drink alcohol - % (n)			
No	34.8 (71)	65.2 (133)	0.474
Yes	30.3 (23)	69.7 (53)	
Smoker - % (n)			
No	32.8 (84)	67.2 (172)	0.380
Yes	41.7 (10)	58.3 (14)	
Continuous medication - % (n)			
No	37.3 (57)	62.7 (96)	0.128
Yes	28.7 (37)	71.3 (92)	
Biochemical profile – median (IR)	**No**	**Yes**	**p**
Serum cortisol	12.9 (6.7)	11.5 (6.2)	0.151
Glycemia	90.5 (15.0)	95.0 (23.0)	0.016*
Total cholesterol	174.0 (52.0)	187.0 (44.9)	0.047*
HDL_cholesterol	68.0 (28.0)	61.0 (28.)	0.007*
LDL_cholesterol	80.0 (35.0)	89.0 (44.0)	0.036*
VLDL_cholesterol	21.0 (17.0)	28.0 (20.0)	<0.001*
Triglycerides	103.0 (84.0)	140.0 (97.0)	<0.001*

BMI: Body Mass Index. Quantitative variables presented in medians and interquartile ranges (IR); categorical variables presented in relative (%) and absolute (n) frequencies. * p-value for the tests: Chi-square or Mann-Whitney U, at 5% significance (p <0.05).

Table 2. CpGs interrelationship by Factor Analysis

	CpGs					
Components	40	41	42	43	44	45
1*	-0.420*	0.636*	-0.045	0.720*	0.047	0.070
2**	0.345	0.287	0.811**	-0.046	0.931**	0.934**

* 1: CpG40-41-43; ** 2: CpG 42-44-45; Kaiser-Meyer-Olkin (KMO): 0.661; Total cumulative variance: 61.8%; Statistical significance: p <0.001 by Bartlett's Test of Sphericity.

Table 3 - Multivariate Poisson regression analysis with robust variance for methylation of NR3C1 1F region

	Methylation								
	Total (CpG40to45)			**Bin 1 (CpG40-41-43)**			**Bin 2 (CpG42-44-45)**		
Variables	**PR**	**95% CI**	**p**	**PR**	**95% CI**	**p**	**PR**	**95% CI**	**p**
Overweight									
No	1			1			1		
Yes	0.60	0.40-0.90	**0.012**	0.62	0.41-0.92	**0.019**	0.23	0.07-0.75	**0.015**
Sex									
Male	1			1			1		
Female	1.34	0.70-2.55	0.382	1.32	0.69-2.53	0.402	0.88	0.25-3.15	0.847
Age (years)	1.00	0.99-1.02	0.647	1.01	0.99-1.03	0.511	0.99	0.92-1.06	0.718
Drink alcohol									
No	1			1			1		
Yes	0.43	0.22-0.85	**0.015**	0.43	0.22-0.86	**0.017**	0.35	0.05-2.68	0.314
Smoker									
No	1			1			1		
Yes	0.89	0.63-1.25	0.485	0.89	0.63-1.25	0.487	0.42	0.16-1.06	0.067
Continuous medication									
No	1			1			1		
Yes	0.91	0.61-1.35	0.632	0.87	0.58-1.30	0.493	0.34	0.06-2.01	0.233
Altered cortisol									
No	1			1			1		
Yes	0.99	0.52-1.90	0.982	1.00	0.52-1.92	0.995	1.26	0.17-9.47	0.821
Altered glycemia									
No	1			1			1		
Yes	1.24	0.82-1.88	0.313	1.24	0.82-1.89	0.308	3.28	0.70-15.34	0.131
Altered lipid profile									
No	1			1			1		
Yes	1.00	0.66-1.51	0.987	1.03	0.67-1.56	0.905	0.20	0.04-0.97	**0.046**
Log pseudolikelihood		-159,23544			-158,25925			-36,294318	
Pseudo R2		0.046			0.044			0.210	
Prob > Chi²		**0.025**			**0.034**			0.040	
Adjustment of models: Hosmer & Lemeshow		**0.99**			**0.99**			0.00	

* PR: prevalence ratio; 95% CI: confidence interval; p: p-value.

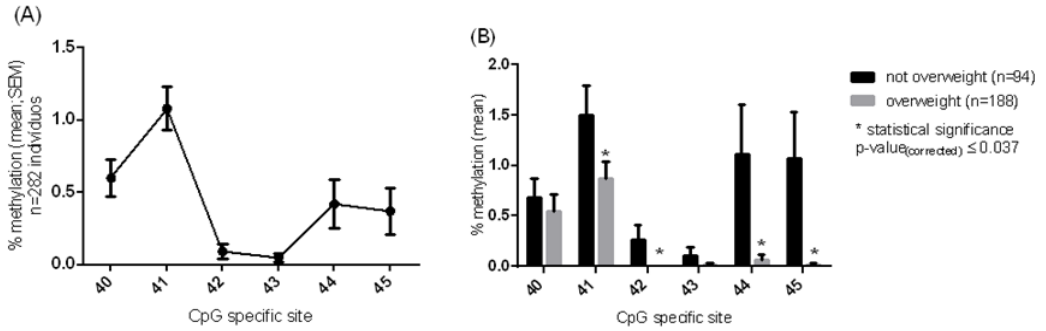

Figure 1 – CpG methylation site-specific in NR3C1 1 F region.

(A) Methylation levels across six CpGs site-specific for the NR3C1 1F region (all study participants). (B) Comparison between non-overweight vs. overweight groups. Non-parametric data are presented in means and standard error of means (SEM) in A and B for improved graphical representation.

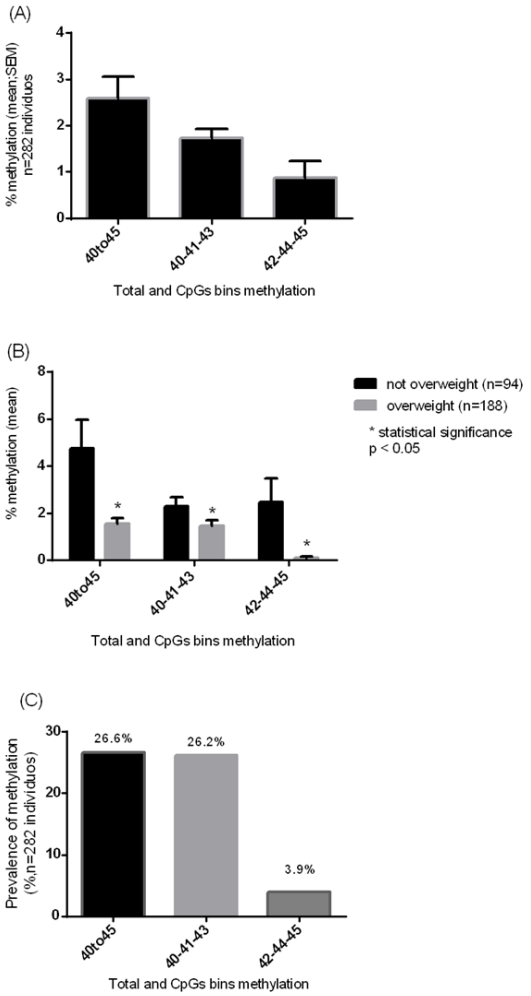

Figure 2: Total methylation and CpGs bins.

(A) Level of total and CpGs bins methylation. (B) Level of total and CpGs bins methylation compared between non overweight and overweight adults. (C) Prevalence of total and CpGs bins methylation. Quantitative methylation (non-parametric data) presented as mean and standard error of mean (SEM) in A and B for improved graphical representation. Qualitative methylation (categorical data) presented as relative frequency (%) in C.

Discussion

To our knowledge, this is one of the first studies to address links between excessive weight accumulation and methylation of the NR3C1 1F region. This cross-sectional investigation sampled 282 individuals, and the study power was 85.7%.[28] We observed a relationship between overweight and hypomethylation of the NR3C1 1F promoter region. In fact, we found that overweight adults had statistically lower levels of total methylation throughout the study region at almost all individual CpG sites, as well as in the interrelations of clustered CpGs (Figures 1 and 2). Our findings also point to a correlation of BMI with hypomethylation at three specific CpGs site and across the interrelation of these sites.

It has been reported that the HPA axis is dysregulated in individuals who experience psychosocial stress and excessive weight gain.[21, 26] However, this relationship appears to be more complex than expected as it involves changes in food intake triggered by compensatory mechanisms in response to stress, particularly stimulus of the nucleus accumbens[4, 29], as well as the leptin system and problems with satiety control.[25, 30, 31]

The HPA axis is recognized to be important for physical and psychological balance and as such is considered integral to the Psycho-Neuro-Endocrine-Immune system.[32, 33, 34] In normal physiological situations, the activated GR-ligand performs the transactivation of genes regulated by the axis[35] and transrepression of inflammation by binding to the transcription factor NFκB, preventing its pro-inflammatory activity[10, 36]

It is likely that the negative crosstalk between both GR-linker and NFkB pathways[5, 10, 37] may indicate an interesting target to be further explored in epigenetic studies involving NR3C1 in the accumulation of weight, stress and inflammation, especially in the low-grade inflammation characteristic of the state obesogenic.

We also observed prevalent methylation in the context of the total segment (Figure 1C) and the interrelationship of methylation percentage between the specific CpG units. From this, it was possible to extract two distinct bins of CpGs within the assessed segment (Table 2), within this, we observed a higher prevalence of methylation in bin 1. This interrelationship has also been observed in another study, in which a coordinated intercorrelation in methylation between CpGs 41 to 47 in adults was found, which was associated with childhood maltreatment.[38]

With reference to previously published studies[15, 38, 39], we observed very low values of methylation (Figure 1A), even in the evaluation of the total segment, corresponding to the sum of the six CpGs (Figure 2A). Low levels of methylation are typical of regions of the genome where the density of CpGs is high, and which are open to regulation by methylation.[40] However, small changes in the percentage of methylation of the GR 1F promoter may be quite relevant, since it is associated with endocrine functional

outcomes[15], as is the case in our study. Findings from other surveys[15, 17, 41] suggest that low levels of methylation in the region, may not interfere with transcription factor binding, but we did not investigate gene expression in this study.

Although NRC31 is a large-scale constitutive gene expressed in the hippocampus, expression in blood cells is seen in B lymphocytes and innate immune cells, however the gene is not expressed in T lymphocytes and monocytes.[8, 14, 42]

It is possible to find differences in methylation in the case of peripheral blood, which contains DNA from several cell types exhibiting different expression patterns. However, there is special interest in peripheral tissues, which are readily accessible in the case of human disease related research related to life adversities, as well as the accumulation of weight and associated comorbidities. In addition, studies of peripheral methylation as a substitute for methylation in hippocampal cells, obtained for example from suicide victims[16], have been supported by suggestions that the methylation patterns in some loci may be the same in the brain and periphery, revealing epigenetic reprogramming related to psychological conditions.[42]

In the present study, after exploring r values found in the Spearman correlations ($r < 0.3$), and in order to seek reinforcement of the findings, we categorized the total methylation data and the CpG bins for the multivariate investigation. Analysis confirmed the relationship between overweight and hypomethylation in the region, through Poisson regressions, with robust variance.

The models showed that hypomethylation of the total segment and of bin 1 was explained by overweight, along with alcohol consumption, when controlled by confounding factors. In bin 2 (model 3), hypomethylation was explained by overweight and an altered lipid profile, also controlled by confounding factors, although the latter model did not show strong adherence. It is important to emphasize that we chose to maintain the covariables in the adjustment of the models, even when they were not associated with overweight or methylation by univariate analysis (data not shown).

The findings also show the importance of the question of obesity; two thirds of the sample consisted of overweight individuals, this group had a higher median age, as well as higher blood glucose levels, triglycerides, total cholesterol and fractions (Table 1). It is possible to summize that the high prevalence of overweight individuals was expected as the study focuses on users of the public health system.[43] In addition, in both developed and developing countries with low mortality, overweight has been listed as a serious major risk factor for noncommunicable disease.[44, 45]

As already stated, this is one of the first studies relating nutritional status to the accumulation of weight and methylation in the 1F promoter region of GR. Interestingly, a previous study evaluated the relationship between the methylation of NR3C1 1F region and

birthweight coupled to placental development, finding a significant correlation between birthweight and mean extent of methylation, and a significant association between GR methylation and large size for gestational age of fetuses.[46] Another group of researchers found hypermethylation of certain CpG sites of the GR gene promoter in women with bulimia, however these findings were in exon 1C.[7]

Finally, we believe that more research is needed to further elucidate the epigenetic mechanisms involved in this complex and multifactorial pathway that encompasses psychosocial stress and excessive weight gain.

Materials and Methods

Participant selection

This cross-sectional study is part of a health research project (PPSUS) with users of the Brazilian universal health system and was carried out according to the principles of the Helsinki Declaration and following a clear explanation of the study protocol, each participant signed a written informed consent form. The study was approved by the Ethics Committee on Human Research, the Health Sciences Center, Federal University of Espirito Santo, under number 1,574,160 issued on June 03, 2016.

Participants were selected on the basis of the following criteria: being in the age group of 20 to 59 years, not being pregnant, having no cognitive conditions that would interfere with the response to the questionnaires, and were required to declare free consent for participation. At first 384 individuals took part, however, a subsample of 282 subjects participated in our biomolecular study of the methylation of NR3C1

The exclusion criteria for the initial sample group were: not consistent with anthropometric data, use of glucocorticoid medication and insufficient biological material for analysis after DNA extraction. The 282 participants were categorized into two groups: non-overweight versus overweight (94 vs. 188 subjects, respectively).

Anthropometric evaluation

The anthropometric evaluation was performed by qualified professionals in the morning, after participants had fasted for a minimum of eight hours, following the technical standards of the Food and Nutritional Surveillance System (SISVAN).[47]

Height was measured using an Alturexata® stadiometer, with a maximum capacity of 2.10 m and an accuracy of 0.5 cm. Body weight was measured using an electronic balance (Tanita®, BC601 model). Body mass index (BMI) was calculated by the quotient between body weight and square height (kg/m2) and classified according to World Health Organisation (WHO) reference for adults[1], in which low weight individuals present BMI

values < 18.5 kg/m^2, eutrophic range from 18.5 to 24.9 kg/m^2, overweight from 25.0 to 29.9 kg/m^2, and obese individuals BMI being ≥ 30.0 kg/m^2. For purposes of dichotomization of the data, we classified participants into two groups: non-overweight (BMI < 25.0 kg/m^2) and overweight (BMI ≥ 25.0 kg/m^2).

Use of continuous medication

All participants were asked about the continued use of the following types of drugs: hypoglycemic agents, antihypertensives, antidepressants, anxiolytics, sleep regulation, contraceptive hormones or thyroid treatment, which are often used by the general population and could interfere with regulation of the HPA-axis and/or in metabolism related to the weight gain. The drugs were subsequently grouped into the variable 'continuous medication', maintaining the dichotomization of response: no/yes.

Blood collection and biochemical analysis

Blood collection was performed by venipuncture in the morning between 7:00 and 9:00 a.m., observing a minimum fast of 8 hours and following protocols of biochemical analyzes of cortisol, fasting glycemia and lipid profile. Cortisol analysis was performed using the chemiluminescence method[48], while the glucose, total cholesterol, high-density lipoprotein cholesterol (HDL-c), and triglycerides analyzes were by enzymatic colorimetry, using specific colorimetric kits (Bioclin®) in an automated biochemical analyzer (Bioclin® BS-120). Low-density lipoprotein cholesterol (LDL-c) was calculated by the Friedewald equation.[49]

Extraction, quantification of genomic DNA and conversion by bisulfite

DNA was extracted from leukocytes per the manufacturer's instructions (QIAamp DNA Mini Kit, Qiagen®).[50] DNA quantity and quality were measured by calculating the DNA concentration and absorbance ratio at 260/280 nm using a NanoDrop®. A ratio between 1.8-2.0 indicated high quality DNA and was the criteria for proceeding to bisulfite conversion.

Sodium bisulfite conversion of 1 µg of DNA from each participant was carried out using the EZ DNA Methylation ™ kit (Zymo Research) according to the manufacturer's instructions. This treatment with bisulfite converts unmethylated cytosines into uracil while the methylated cytosines remain intact.

PCR amplification and pyrosequencing

Amplification by Polymerase Chain Reaction (PCR) and pyrosequencing were adapted from previous studies.[17, 39, 51, 52] The NR3C1 1F region, which has 47 CpGs

sites was amplified using forward and reverse primers, which spanned the gene region: 961 to 1371 (sequence data submitted to the GenBank database whith accession number AY436590.1), fragment with 410 base pairs. PCR was performed using HotStart Taq DNA Polymerase (Qiagen®) with 20 ng of bisulphite-treated DNA template per reaction. The quality of the PCR product and lack of contamination were confirmed on 2% agarose gels using GelRedTM (Uniscience).

The PCR products were purified and sequenced using the pyrochemical PSQ96ID (Qiagen®, Valencia, CA) with the reagent kit PyroMark Gold Q96 (Qiagen®, Valencia, CA) according to the manufacturer's protocol. Amplification primers, PCR conditions and sequencing primers are described in Table 4. Within the 1F region, it was possible to sequencing and analyzing the methylation of six CpGs sites 40 to 45 as shown in Figure 3.

Table 4 – PCR conditions and primer sequences for the pyrosequencing reaction

PCR Primer		**PCR conditions**		
Forward	5'-TTTTTTTTTTGAAGTTTTTTTA-3'	95 ºC	(14'30'')	
Reverse	5'-BIOTIN-CCCCCAACTCCCCAAAAA-3'	94 ºC	(30'')	
		50 ºC	(30'')	45 cycles
		72 ºC	(30'')	
		72 ºC	(10')	
		4 ºC	indefinitely	
Sequencing primer				
40 to 42 CpG	5'-AGAAAAGAAATTGGAGAAATT-3'			
43 to 45 CpG	5'-GTTTTAGAGAGATTAGGT-3'			

Figure 3. NR3C1 1F region.

Representative scheme of the amplified region of 47 CpGs and the six CpGs site-specific analyzed using bisulfite-pyrosequencing assays (red box). To the left (in blue), numbering extracted according to the GenBank database (Access number: AY436590.1).

Statistical analysis

Data were tabulated and submitted for consistency analysis. We excluded from the analysis individuals who did not present consistent anthropometric data and NR3C1 1F region methylation, in addition to those who reported use of corticosteroid medications. The research was performed with 282 participants, categorized into two groups: non-overweight (94 individuals) and overweight (188 individuals). This sub-sample did not differ from the initial PPSUS sample regarding the prevalence of overweight or any other covariates such as sex, age, lifestyle, cortisol, glycemia and lipid profile.

Data were examined for normality using the Kolmogorov-Smirnov normality test. To characterize the samples by overweight, the categorical data were presented at relative and absolute frequencies and compared by chi-square test. Continuous variables were presented in median and interquartile ranges and compared by the Mann-Whitney U test. Spearman correlation was also tested to verify correlation between methylation and BMI.

Total methylation of the segment (CpG40to45), as well as the median percentage of methylation of each specific CpG site were analyzed. In this case, we used the 5% significance, corrected by the Benjamini-Hochberg method[53] to control the false discovery rate (FDR), with a corrected p-value equal to or less than 0.037, in the case of comparisons between groups, and corrected p-value equal to or less than 0.017, in the case of Spearman correlation analysis.

The methylation data of the six CpGs specific sites were subject to factorial analysis in order to verify inter-relationship between the CpGs of the analyzed segment. We use the method of main components extraction by advantage of non-normality assumption of the variables involved. The analysis of the quality of fit of the model was performed using Kaiser-Meyer-Olkin criterion (KMO) and, in Bartlett's Sphericity Test, a significance of 5% ($p < 0.05$) was considered.[54] The matrix of factor loads was estimated and, after that, the orthogonal rotation varimax was performed.

After extraction of factors, CpGs bins were defined and we proceeded with the analysis, with the tests of comparison between groups non-overweight and overweight, Spearman correlations between BMI and methylation and, later, Poisson regression with robust variance from the categorization of methylation data dichotomously in: non-methylated (0.1%) and methylated (≥ 0.1%).

Statistical analyzes were performed using SPSS® software, version 15.0 for Windows (IBM®) and, in the case of Poisson regression, the STATA® software, version 9.0 (StataCorp® LP, College Station) was used. For graphical presentation of the results, GraphPad Prism®, version 7.0 (GraphPad® Software Inc.) was used, and for the best visualization, we constructed graphs from the values of mean and standard errors of mean (SEM).

Regression analysis, modeling and treatment of covariates

Multivariate Poisson regression analyzes with robust variance were performed to test if overweight was associated with the methylation of NR3C1 1F region, and if covariables also had predictive methylation ability.

Three different multivariate models were tested, with the following outcome variables: total methylation (Model 1), methylation of CpGs bin 1 (Model 2) and methylation of CpGs bin 2 (Model 3). In addition to the main variable of interest (overweight), all covariates were included: sex, age, alcohol, smoker, continuous medication, altered cortisol, glycemia, and lipid profile, due to their direct or indirect association with obesity and, or with the activation of the HPA axis.[2, 6, 14, 25, 55] To verify the final adherence of the model, an adjustment was made using the Hosmer & Lemeshow test. The measure of effect was given by the prevalence ratio with a 95% confidence interval.[56] The level of significance considered in the model analysis was 5%, and after Benjamini-Hochberg's correction[53], the variables that had explanatory capacity were those that presented p-value equal to or less than the FDR correction, being 0.015, 0.019 and 0.046 for the respective models 1, 2 and 3.

With the exception of age, the other covariates were dichotomized. Total cholesterol, its fractions and triglycerides were grouped into lipid profile, with the variable also dichotomized as normal or altered.

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Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed. The funders had no role in the study design, data collection, analysis, decision to publish, or preparation of the manuscript.

Author Contributions

Conceived and designed the experiments: FVF, AMAS. Performed the experiments: FVF, JAP, ARB, JKA, CLC, JGS, ZTG, WMB, LMRBA, BPS. Analyzed the data: FVF, AMAS, WMB, IDL. Contributed reagents/materials/analysis tools: FVF, JAP, ARB, CLC, JKA, WMB, JGS, LMRBA, BPS, ZTG, IDL, AMAS. Wrote the paper: FVF, AMAS. Research Project Coordinator: AMAS.

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4. Conclusões e considerações finais

4.1. Conclusões

- Os indicadores de estresse que tiveram associação com a adiposidade abdominal no meio rural e urbano foram respectivamente: cortisol sérico (com associação inversa) e pressão arterial alterada, reforçando a influência do meio local e psicossocial na modulação do estresse e da resiliência aos agentes estressores;

- Foram verificadas altas prevalências de sobrepeso e obesidade na população frequente ao SUS, bem como de risco de complicações cardiometabólicas relacionadas à obesidade abdominal;

- A análise do perfil sociodemográfico, de saúde e estilo de vida de adultos, usuários do da Rede Municipal de Atenção Básica do SUS, demonstrou que se trata de uma população com alta prevalência de Insegurança Alimentar e Nutricional, baixa renda e baixo nível de escolaridade, sendo estes últimos dois fatores mais agravantes no meio rural. As condições de saúde observadas foram preocupantes, tanto pela alta proporção de indivíduos com excesso de peso, quanto pelo descontentamento da saúde em geral, além da alta prevalência de importantes indicadores de estresse psicossocial, em especial: cortisol baixo, depressão e pressão arterial alterada;

- O excesso de peso esteve relacionado com a hipometilação da região promotora do gene do receptor do glicocorticoide (NR3C1 1F região) nas diversas formas de análises realizadas: no segmento total, na maioria das CpGs sítio específicos e nos bins de CpGs extraídos;

- A prevalência de metilação de NR3C1 1F região na amostra estudada foi considerável, apesar dos percentuais de metilação das CpGs sítio específicos terem sido baixos;

- A análise do perfil de metilação no segmento apontou para intercorrelação entre CpGs sítio específicos, com a extração de componentes principais;

4.2. Considerações finais

Os resultados deste estudo estreitam ainda mais a relação entre estresse e acúmulo de peso, via eixo HPA, e apoiam a hipótese da bidirecionalidade. Sugere-se o hipocortisolismo como biomarcador da adiposidade abdominal e o excesso de peso como consequência do estilo de vida atual, influenciando na modulação epigenética.

Considerando que este é o primeiro estudo a investigar a relação entre excesso de peso e metilação da região promotora 1F do GR, os resultados indicam um novo caminho a ser aprofundado no contexto das investigações relacionadas ao acúmulo excessivo de gordura e seus desdobramentos frente às alterações epigenéticas.

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6. Anexos: Comprovantes de submissão de artigos científicos

Gmail

Flávia Vitorino <flavitorino@gmail.com>

Submission Confirmation for Overweight is associated with hypomethylation of the glucocorticoid receptor gene (NR3C1 1F region) promoter region

1 mensagem

Epigenetics <em@editorialmanager.com> 28 de maio de 2018 15:36
Responder a: Epigenetics <kepi-peerreview@journals.tandf.co.uk>
Para: Flávia Vitorino Freitas <flavitorino@gmail.com>

Dear Mrs Freitas,

Your submission entitled "Overweight is associated with hypomethylation of the glucocorticoid receptor gene (NR3C1 1F region) promoter region" has been received by Epigenetics.

You will be able to check on the progress of your paper by logging on to Editorial Manager as an author at https://epi.editorialmanager.com/.

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Thank you for submitting your work to this journal.

Kind regards,

Epigenetics

Gmail Flávia Vitorino <flavitorino@gmail.com>

PLOS ONE: Notification of co-authorship on manuscript - [EMID:98c12183e56b8f94]

1 mensagem

PLOS ONE <em@editorialmanager.com> 4 de maio de 2018 18:02
Responder a: PLOS ONE <plosone@plos.org>
Para: Flavia V Freitas <flavitorino@gmail.com>

PONE-D-18-13489
Psychosocial stress and central adiposity: A Brazilian study with users of the public health system
Dr. Iuri Drumond Louro

Dear Flavia Freitas,

You are receiving this email because you have been listed as an author on a manuscript recently submitted to PLOS ONE and entitled "Psychosocial stress and central adiposity: A Brazilian study with users of the public health system".

The corresponding author for the submission process is: Dr. Iuri Drumond Louro
The full author list for the submission is: Flavia V Freitas; Wagner Barbosa; Laiz Silva; Mariana Garozi; Julia Pinheiro; Aline Borçoi; Catarine Conti; Juliana Arpini; Heberth Paula; Mayara Oliveira; Anderson Archanjo; Erika Freitas; Daniela Oliveira; Elizeu Borloti; Iuri Drumond Louro, M.D, Ph.D.; Adriana Alvares-da-Silva

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1 mensagem

PLOS ONE <em@editorialmanager.com> 28 de abril de 2018 15:04
Responder a: PLOS ONE <plosone@plos.org>
Para: Flavia Freitas <flavitorino@gmail.com>

PONE-D-18-12851
Food and Nutritional Insecurity: epigenetic correlation among BDNF gene, social and health status in familiar coffee farmers
Dr. Iuri Drumond Louro

Dear Flavia Freitas,

You are receiving this email because you have been listed as an author on a manuscript recently submitted to PLOS ONE and entitled "Food and Nutritional Insecurity: epigenetic correlation among BDNF gene, social and health status in familiar coffee farmers".

The corresponding author for the submission process is: Dr. Iuri Drumond Louro
The full author list for the submission is: Wagner Barbosa; Carlos Almança; Flavia Freitas; Catarine Conti; Julia Pinheiro; Alcemi Barros; Joaquim Santos; Aline Borçoi; João Simão; Luciane Cardoso; Juliana Arpini; Mayara Oliveira; Juliana Dalbo; Anderson Archanjo; Suzanny Mendes; Lucas Maia; Iuri Drumond Louro, M.D, Ph.D.; Adriana Alvares-da-Silva

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January 10, 2018

Title: "**Interaction between methylated and non-methylated status within the exonic 1F region of the *NR3C1* gene**"
Tracking #: 2018-00430
Author(s):
Catarine Conti (Federal University of Espírito Santo)
Flavia Vitorino Freitas (UFES)
Júlia de Assis Pinheiro (UFES)
Aline Ribeiro Borçoi (UFES)
Juliana Krüger Arpini (UFES)
Lidia Maria Rebolho Batista Arantes (Barretos Cancer Hospital)
Bruna Pereira Sorroche (Barretos Cancer Hospital)
Adriana Madeira Álvares-da-Silva (UFES)

Dear Dr. Freitas,

"**Interaction between methylated and non-methylated status within the exonic 1F region of the *NR3C1* gene**," for which you participated as an author, was submitted by Dr. Conti and received in our office on January 9, 2018. The manuscript has been assigned tracking number 2018-00430.

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