UNIVERSIDADE ESTADUAL PAULISTA "JÚLIO DE MESQUITA FILHO"
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INFLUÊNCIA DOS CARETONÓIDES, RETINOL E α-TOCOFEROL E DOS POLIMORFISMOS DOS GENES *CYP1A1*, *GSTP1*, *MTHFR* (*A1298C* E *C6777*) E *XRCC1* (*194Trp* E *399Gln*) SOBRE OS NÍVEIS DE DANOS OXIDATIVOS NO DNA, DE URACILAS INCORPORADAS AO DNA E DA CAPACIDADE DE REPARO DO DNA

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Dedicatória

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RESUMO

É crescente o número de estudos que demonstram a importância de micronutrientes e compostos bioativos presentes nos alimentos na prevenção de diversas doenças degenerativas crônicas. Entretanto vários estudos moleculares epidemiológicos têm demonstrado que além de fatores ambientais, como a dieta, essas doenças degenerativas podem ser modulada por genes envolvidos no biometabolismo de xenobióticos, metabolismo do carbono e no reparo de DNA. Portanto, o presente estudo avaliou a possível influência do padrão alimentar e dos polimorfismos dos genes *GSTP1*, *CYP1A1*, *XRCC1* e *MTHFR* sobre os níveis de danos oxidativos no DNA, uracilas incorporadas no DNA e eficiência do sistema de reparo de DNA em dois grupos de indivíduos residentes em Botucatu com diferentes padrões alimentares. Grupo I (GI): 87 indivíduos com alimentação rica em produtos orgânicos, grãos integrais, frutas e vegetais, e baixa ingestão de produtos industrializados; Grupo II (GII): 97 indivíduos com alimentação rica em produtos industrializados e pobres em frutas e vegetais. A quantificação do nível de danos oxidativos no DNA, uracilas incorporadas ao DNA e a eficiência do sistema reparo de DNA em linfócitos de sangue periférico, foi analisada utilizando-se o Teste do Ensaio Cometa. Os polimorfismos dos genes *GSTP1, CYP1A1*, *XRCC1* e *MTHFR* foram analisados por real-time PCR. Também foi realizada a análise dos níveis de luteína, criptoxantina, α-caroteno, β-caroteno, licopeno, retinol e α-tocoferol no plasma, pela técnica de cromatografia líquida de alta pressão (HPLC). Os indivíduos do GI apresentaram menores níveis de danos oxidativos no DNA e menores níveis de dano no DNA induzidos pela H_2O_2 quando comparados aos indivíduos do GII. Quanto aos subgrupos de micronutrientes: Indivíduos do subgrupo percentil 75 para todos os micronutrientes tiveram maior nível de danos no DNA do que os indivíduos do percentil 50 e do que os indivíduos do subgrupo percentil 25 apenas para β-caroteno, retinol e α-tocoferol. Além disso, os indivíduos do subgrupo percentil 25 de luteína, criptoxantina e α-caroteno tiveram nível mais elevado de danos no DNA do que indivíduos do percentil 50. Indivíduos do subgrupo percentil 25 para todos os micronutrientes tiveram maiores níveis de purinas oxidadas e maiores níveis de pirimidinas oxidadas para a luteína do que os indivíduos do percentil 50. Além disso, os indivíduos do percentil 25 de luteína, α-caroteno e β-caroteno tiveram maiores niveis de purinas oxidadas e pirimidinas oxidadas (apenas para luteína) em relação a indivíduos do percentil 75. Indivíduos do subgrupo percentil 75 de luteína, licopeno, α-caroteno, β-caroteno, retinol e α-tocoferol tiveram maior nível de uracilas incorporadas ao DNA do que os indivíduos do percentil 50 e um maior nível de uracilas incorporadas do que os indivíduos do percentil 25

para retinol. Indivíduos do percentil 75 de α-tocoferol e luteína tiveram maior nível de danos no DNA induzidos por H_2O_2 e menor capacidade de reparo do DNA do que os indivíduos do percentil 50, respectivamente. Com relação aos indivíduos de GI: a) portadores do genótipo *GSTP1 AG* apresentaram níveis mais elevados de danos no DNA induzidos por H2O2, b) portadores do genótipo *XRCC1 194 CC* apresentaram maiores níveis de quebras de fita simples e dupla e sítios alcali-lábeis, c) portadores dos genótipos *XRCC1 194 CC* e *XRCC1 194 TC* apresentaram menor capacidade de reparo do DNA, e d) portadores dos genótipos *XRCC1 399 GG* e *XRCC1 399 AA* apresentaram maiores níveis de pirimidinas oxidadas. Com relação aos indivíduos do GII: a) portadores dos genótipos *XRCC1 194 TT* e *XRCC1 194 TC* apresentaram maiores níveis de pirimidinas oxidadas, b) portadores dos genótipos *XRCC1 399 GG* e *XRCC1 399 AA* apresentaram maiores níveis de uracila incorporada ao DNA. As análises de nossos resultados fornecem evidências de que uma dieta rica em grãos integrais, frutas e legumes e pobre em produtos industrializados pode proteger contra danos oxidativos no DNA. No entanto, é importante ter uma ingestão adequada de nutrientes, pois tanto a deficiência quanto o excesso de micronutrientes podem levar a aumento de danos no DNA. Além disso, os polimorfismos de genes de metabolismo de xenobióticos (*GSTP1*) e reparo de DNA (*XRCC1 194Trp e 399Gln*) podem modular os níveis de danos no DNA e a eficiência do sistema de reparo de DNA.

REVISÃO BIBLIOGRÁFICA

I – INTRODUÇÃO

I.1 – Considerações Iniciais

Revendo o desenvolvimento histórico da relação entre dieta e saúde, observa-se que no início do século XX a ciência nutricional estava relacionada à higiene e transformação dos alimentos (Müller e Kersten, 2003). Posteriormente, com o auxílio da Bioquímica e da Fisiologia, foram descritas as vitaminas e entendido o metabolismo energético (Müller e Kersten, 2003). Durante a segunda metade do século, com a ampliação dos conhecimentos de biologia celular e molecular, houve maior preocupação com a otimização da dieta, com especial ênfase à ingestão balanceada de micro e macronutrientes (Fenech, 2010). Esse avanço levou ao aumento da produção de suplementos dietéticos e contribuiu para o aumento da expectativa e qualidade de vida (Ommen, 2007).

Atualmente, a aplicação das técnicas de biologia molecular e o êxito do Projeto Genoma Humano abriu uma nova era nas pesquisas nos campos da Medicina e da Nutrição, dando origem a Nutrigenética e a Nutrigenômica ou Genômica Nutricional (Fenech et al., 2011). Essa nova ciência, que trata das interações nutriente-gene e suas implicações para a saúde humana, vêm em crescente desenvolvimento dado à importância da dieta personalizada (Fenech et al., 2011). Mais especificamente, a Nutrigenômica estuda como os constituintes da dieta interagem com os genes e seus produtos para alterar o fenótipo e, inversamente, como os genes e seus produtos metabolizam micronutrientes e compostos bioativos presentes na dieta (Fenech et al., 2011). Portanto, os dados gerados em pesquisas de Nutrigenômica fornecem informações importantes de como aperfeiçoar a ingestão de

nutrientes com o objetivo de prevenir ou retardar uma série de doenças (Fenech et al., 2011).

A desnutrição causada pela deficiência calórico-protéica e de nutrientes tem se caracterizado como sério problema em países em desenvolvimento (Anselmo et al., 2009). A carência de determinados micronutrientes (sendo os principais, o iodo, o ferro, o folato, o cálcio e as vitaminas A e D) pode provocar desde a diminuição na imunidade, osteoporose, cegueira, bócio, anemia, diminuição da capacidade de aprendizado, letargia, retardo mental, até a morte (Fenech & Fergunson, 2001). Algumas doenças que estão relacionadas à alimentação inadequada e que têm apresentado grande repercussão mundial merecem destaque: a osteoporose, doenças degenerativas cardiovasculares, mal de Alzheimer, envelhecimento precoce e câncer (Fenech & Fergunson, 2001; Aruoma, 2003; Voutilainen et al., 2006; Mmbaga & Luk, 2012).

O alto índice de neoplasias observado atualmente pode ser reflexo das mudanças ocorridas nos hábitos alimentares com o advento da era tecnológica. Vários aditivos químicos alimentares têm atividade carcinogênica comprovada (Griciute L, 1979; Anwar-Mohamed & El-Kadi, 2007), contudo, não só a ingestão aumentada desses produtos, mas, também, a diminuição do consumo de alimentos naturais são fatores que contribuem para o aparecimento das doenças da vida moderna (Trichopolou *et al*., 2003). Diversos estudos têm demonstrado maior expectativa de vida e menor risco para o desenvolvimento de câncer e de doença isquêmica do coração em indivíduos que optam por dietas ricas em vegetais (IARC, 2003; Trichopolou *et al*., 2003; Loft *et al*, 2008). No entanto, o mecanismo de ação desses micronutrientes é complexo e ainda não totalmente compreendido (Loft *et al*, 2008).

Em 2002, um estudo epidemiológico demonstrou, por exemplo, que indivíduos que consumiam grande quantidade de frutas e vegetais ricos em micronutrientes com

propriedades antioxidantes, como as vitaminas C e E, carotenóides e flavonóides, apresentavam menor incidência de câncer de pulmão, estômago, mama, cólon, faringe e fígado (Moller & Loft, 2002). Por outro lado, Ferguson (2002) mostrou que aditivos químicos utilizados na conservação de alimentos (componentes N-nitrosos) e aminas heterocíclicas e hidrocarbonetos aromáticos policíclicos (PAH) produzidos durante o preparo de carnes em altas temperaturas podem aumentar o risco para o desenvolvimento de câncer de cólon e de próstata.

Portanto, torna-se cada vez mais evidente que a nutrigenética e nutrigenômica tem um papel central na investigação dos efeitos da nutrição sobre os resultados da saúde, e dos impactos dos nutrientes que podem ser muito bem avaliados por uma multidão de tecnologias 'omics' e biomarcadores (Fenech et al., 2011). Dentre estes biomarcadores, danos no DNA tem ganhado destaque, pois são um biomarcador claro do risco de desenvolvimento de patologia, uma vez que eles podem ser atenuados através da promoção de apoptose de células geneticamente anormais ou através da redução da taxa de acúmulo de danos do DNA (Fenech et al., 2011). Deste modo, a significância dos danos no DNA como um precursor de doenças crónicas degenerativas está bem estabelecida (Azqueta & Collins, 2012).

I.2 – Micronutrientes e danos no DNA

Os carotenóides constituem uma família de mais de 600 compostos já identificados na natureza, sendo que cerca de 50 apresentam atividade pró-vitamínica A, ou seja, são precursores da vitamina A em mamíferos (Olson,1989). Dentre esses, o β-caroteno é o mais abundante na natureza e é encontrado em vegetais e frutas de cor verde-escuro e amarelo-alaranjado (Mangels *et al*.,1993; Rock *et al.*,1996). Estudos *in vitro*, que avaliaram os

efeitos dos carotenóides sobre danos induzidos no DNA de células humanas demonstraram, por exemplo, que β-caroteno diminuiu os danos gerados pelo ácido peroxinitroso em células leucêmicas (Hiramoto et al., 1999) e o licopeno diminuiu lesões oxidativas em células de hepatoma (Park et al., 2005) e em melanócitos (Smit et al., 2004). Existem inúmeros outros relatos na literatura sobre a existência de correlação direta entre a ingestão e/ou níveis séricos de vitamina C, vitamina E e carotenóides e a proteção contra certos tipos de câncer (Block,1991; Knekt,1993; Flagg *et al.*,1995; Eichholzer *et al.,*1996; Ziegler *et al*.,1996a; Patterson *et al.*,1997). Foi observado, também, que o ácido ascórbico, o α-tocoferol e o β-caroteno são mais efetivos quando combinados, visto que esses micronutrientes podem interagir no ambiente celular e potencializar a defesa antioxidante (Niki *et al*.,1995) protegendo contra danos oxidativos no DNA (Anderson,1996; Cozzi *et al*.,1997; Pool-Zobel *et al.*,1997).

Vários estudos *in vitro* têm demonstrado que alguns micronutrientes presentes na dieta (carotenóides e vitaminas) têm um potencial antioxidante, demonstrando um efeito protetor contra o câncer, uma vez que eles atuam como a primeira linha de defesa celular contra a oxidação do DNA (Azqueta & Collins, 2012). Eles atuam como antioxidantes por serem eficientes seqüestradores de vários radicais livres (Miller et al., 1996; Collins et al., 2001). Alem disso, o acúmulo de evidências tem confirmado que vários micronutrientes (vitaminas e minerais) atuam como parte importante da estrutura de proteínas (p.ex., metaloenzimas) e são cofatores enzimáticos nas reações de síntese, reparo e metilação do DNA, prevenção de danos oxidativos no DNA, manutenção de telômeros, além de atuarem no processo de apoptose (Fenech, 2008; Fenech, 2010). Estudos recentes também estão descrevendo que os carotenóides podem mediar os seus efeitos através de outros mecanismos, tais como na comunicação entre as células (gap-junction), na expressão de genes supressores de tumores, na carcinogênese, na proteção do DNA na regulação do

crescimento celular, na resposta imune, na proteção contra a peroxidação de lípidos e, como moduladores de enzimas metabolizadoras de fase I e II, modulando a expressão de genes (Paiva et al., 1999; Tapiero et al., 2004; Rao et al., 2007; Aydin et al., 2012). No entanto, a suplementação de carotenóides ainda apresenta resultados que são incertos e controversos. Por exemplo, os resultados de estudos envolvendo suplementação de β-caroteno foram conflitantes, onde alguns não apresentaram efeito, outros relataram uma diminuição ou aumento de incidência de câncer (Haegele et al., 2000). Portanto, a evidência de uma ação protetora dos carotenóides contra o câncer em humanos pode ser, no mínimo, um erro (Collins et al., 2001). Isso ocorreu talvez devido à administração desbalanceada de suplementos ou ingestão elevada. Tanto a deficiência de micronutrientes, quanto o excesso podem causar danos genômicos que são equivalentes a níveis de danos genômicos causados pela exposição a doses significativas de agentes genotóxicos ambientais, tais como carcinogênicos químicos e radiação ultravioleta e ionizante (Fenech, 2010).

De acordo com Bruce Ames (2001) as deficiências de micronutrientes como o ácido fólico, as vitaminas B12, B6, C e E, niacina, ferro e zinco podem mimetizar o efeito da radiação ou de agentes químicos na indução de danos ao DNA. A deficiência de ácido fólico em seres humanos tem sido relacionada à anemia megaloblástica, a defeitos de fechamento do tubo neural em neonatos, a doenças cardíacas e ao desenvolvimento de câncer, especialmente do cólon e reto (Duthie, 1999). Há evidências demonstrando que em condições de deficiência de ácido fólico, a uracila é incorporada erroneamente ao DNA no lugar da timina (Eto & Krumdieck, 1986), e que a incorporação excessiva dessa base pode não só gerar mutações de ponto, mas, também, quebras de fitas simples e duplas no DNA, podendo levar a aberrações cromossômicas (Blount & Ames, 1995; Blount *et al*., 1997). Foi descrito, também, que a redução do nível de folato em culturas de linfócitos é responsável por danos no DNA equivalentes àqueles induzidos por 0,2 Gy de radiação

ionizante, dose cerca de 10 vezes maior que a dose anual máxima permitida a indivíduos que trabalham com radiação (Fenech, 2008). Havia sido previamente relatado que tanto o ácido fólico como a vitamina B12 são importantes para a síntese de metionina e S-adenosil metionina (SAM), compostos esses necessários para a manutenção dos padrões de metilação do DNA e, portanto, da regulação da expressão gênica (Duthie, 1999). Além disso, segundo Fenech (2007) os danos genômicos causados por deficiência moderada de micronutrientes são equivalentes a aqueles causados pela exposição a agentes genotóxicos ambientais, tais como alguns carcinógenos químicos e radiações ultra-violeta (UVA) e ionizante. Variações na ingestão de micronutrientes podem, portanto, estar relacionadas a eventual instabilidade genômica, a qual pode ser detectada como mutação de ponto, quebra cromossômica e segregação cromossômica errônea. Variações na ingestão de micronutrientes também podem levar a alteração na expressão gênica, estresse oxidativo, necrose e apoptose (Fenech & Ferguson, 2001). Embora não haja consenso sobre o nível de micronutrientes necessários para prevenção de danos no DNA, admite-se que cerca de 40 micronutrientes, entre vitaminas, minerais essenciais e outros componentes, são requeridos em pequenas quantidades na dieta humana para um metabolismo celular eficiente (Ferguson, 2002).

Por outro lado, alguns estudos recentes têm mostrado que carotenóides em concentrações elevadas podem causar efeitos pró-oxidantes (El-Agamey et al., 2004). Por exemplo, experimentos com β-caroteno, em excesso, mostraram aumentos de quebras de fitas de DNA (SBs) (Yurtcu et a., 2011), fragmentação de DNA (Jang et al., 2009), e purinas oxidadas (van Helden et al., 2009; Bergström et al., 2011). Além disso, num outro estudo, usando suplementação com β-caroteno em fumantes, foi encontrado um aumentado da mortalidade por câncer de pulmão, quando comparado com o grupo que não recebeu a suplementação de β-caroteno (The Alpha-Tocopherol, Beta Carotene Cancer Prevention

Study Group, 1994). Portanto, é fundamental, nestas áreas, caracterizar a ingestão dietética ideal para a prevenção de danos no DNA e expressão inadequada de genes para subgrupos genéticos e para cada indivíduo, uma vez que a quantidade de micronutrientes que parecem proteger contra os danos do genoma varia muito entre os tipos de alimentos, e uma escolha cuidadosa é necessária para projetar padrões dietéticos ou valores de referência dietéticos para otimizar a manutenção da saúde genômica (Bull e Fenech, 2008).

I.3 – Dieta e Polimorfismos

Sabe-se que não apenas os fatores ambientais estão relacionados à indução de danos e mutações no DNA, mas que existe uma suscetibilidade genética relacionada à maior ou menor ocorrência desses danos (Bull C, Fenech M, 2008).

De acordo com o Conselho Norte-Americano de Pesquisa, os "marcadores de suscetibilidade" são definidos como diferenças biológicas, entre indivíduos ou populações, capazes de afetar a resposta do organismo a agentes ambientais. Entre os marcadores de suscetibilidade mais significativos, estão as diferenças genéticas na capacidade de reparo de danos no DNA, na dinâmica das reações do biometabolismo de agentes xenobióticos (Louro, 2002) e no metabolismo do carbono (Midttun et al., 2012).

I.3.1 – Metabolismo de Xenobióticos

O sistema de metabolização xenobiótica humano compreende duas classes de enzimas: as de metabolismo oxidativo mediado ou de fase I e as enzimas conjugadas ou de fase II. O objetivo final de ambas as fases da metabolização xenobiótica é aumentar a solubilidade em água dos compostos, facilitando assim sua excreção do organismo. Os aditivos químicos presentes no meio ambiente e na dieta necessitam de ativação metabólica para se tornarem metabólitos altamente reativos capazes de ligarem-se ao DNA e exibirem atividade carcinogênica. Esta ativação é feita pelas enzimas da fase I, que são principalmente enzimas da superfamília Citocromo P450 (CYPs) (Nebert, 1991; Perera, 1996; Shimada e Fujiikuriyama, 2004). As CYPs catalisam mais de 20 diferentes tipos de reações, incluindo a hidroxilação de carbono; oxigenação heteroátomo; libertação heteroátomo ou desalquilação e epoxidação (Avadhani et al., 2011). Algumas das mais incomuns reações catalisadas por CYPs são a redução, dessaturação, clivagem de éster oxidativo; expansões em anel e formação de anel (Avadhani et al., 2011). Enzimas do Citocromo P450 são as principais enzimas na ativação metabólica dos PAH, que são um dos principais carcinógenos encontrados na carne grelhada a altas temperaturas (Strange et al., 1999; Sugimura et al., 2004).

Os aditivos químicos em sua maioria necessitam ser biotransformados para eliminarem seus efeitos carcinogênicos. (Taningher *et al*., 1999). As Glutationa S-transferases (GSTs) são enzimas de fase II que estão envolvidas nas reações de conjugação e detoxificação de vários xenobióticos, e agem então como enzimas inativadoras dos produtos da fase I, tornando os metabólitos resultantes das reações de fase I mais hidrofílicos e de fácil excreção (Tijhuis et al., 2007). Um polimorfismo genético que aumente a expressão das enzimas de fase I pode aumentar a quantidade de

carcinógenos reativos formados e, então, aumentar o risco de desenvolvimento de câncer (Dunning *et al*., 1999). Desta forma, os genótipos que conferem variações nos níveis de atividade enzimática das fases I e II estão associados à suscetibilidade individual ao desenvolvimento de câncer (Wu et al., 2004).

Recentemente, especial atenção tem sido direcionada na tentativa de elucidar as bases moleculares dos polimorfismos de genes que codificam enzimas envolvidas na ativação e detoxificação de agentes xenobióticos. Diversos investigadores têm estudado a interação entre os genótipos referentes a genes de biometabolismo e alguns tipos de alterações genéticas; como danos no DNA e micronúcleo, que há vários anos têm sido usados como marcadores de exposição genotóxica e efeitos precoces de carcinógenos genotóxicos (Norppa, 2004).

I.3.1.1 Polimorfismos dos genes da Fase I

I.3.1.1.1 *CYP1A1*

O gene *CYP1A1* está mapeado no cromossomo 15q22-24 (Hildebrand *et al*., 1985; Vibhuti et al., 2010) e metaboliza PAH, aminas heterocíclicas aromáticas e hidrocarbonetos aromáticos polihalogenados entre outros. Estes compostos têm afinidade pelo receptor do aril hidrocarbono, que por sua vez induz a ação da família CYP1, que os metaboliza, transformando-os em produtos carcinogênicos (Nebert e Dalton, 2006; Sangar et al., 2010). Inicialmente foram descritos nove alelos polimorfos, dos quais quatro vêm sendo analisados quanto a uma possível associação à susceptibilidade ao câncer, que são: *CYP1A1*2A, *2B, *3* e *4*. O alelo denominado *CYP1A1 G* apresenta uma guanina no lugar de uma adenina na região 3' flanqueadora

do gene *CYP1A1* (462A para G), dando origem a um sítio de restrição *Msp*II (Vibhuti et al., 2010). Em vários estudos, os polimorfismos do gene *CYP1A1* foram associados com níveis mais elevados de danos genotóxicos e risco de desenvolvimento de câncer [Shen et al., 2005]. O alelo *CYP1A1* variante (alelo G) mostrou também propriedades reguladoras diferenciais na indução de expressão e de cinética enzimática em comparação com o tipo selvagem (alelo A) (Butkiewicz et al., 1998, Georgiadis et al., 2005), embora algumas discrepâncias permanecem entre as referências. Por exemplo, Schwarz et al. (2001) relataram uma menor atividade enzimática da forma variante *CYP1A1 Ile462Val* (*CYP1A1 AG* e *GG*), quando comparado com o tipo selvagem (*CYP1A1 AA*), mas estes resultados discordam dos apresentados por Kawajiri et al. (1993), que encontrou uma maior atividade na forma variante (*CYP1A1 GG*). Embora, CYP1A1 seja importante para a ativação de pré-carcinogenos (Ingelman-Sundberg, 2001), cuja enzima codificada está envolvida na bioativação e detoxificação de uma variedade de xenobióticos presentes nos alimentos [Canalle et al., 2004], os resultados encontrados na literatura ainda são contraditórios. Alguns estudos apontam para um significante aumento do risco de câncer oral observado para indivíduos com genótipo homozigoto (*CYP1A1 GG*) (Cha *et al*., 2007), mas outros estudos não verificaram associação entre o *CYP1A1* e o risco de câncer, principalmente o de mama (Bailey *et al*., 1996, Singh *et al*., 2007).

I.3.1.2 Polimorfismos de genes da Fase II

I.3.1.2.1 *GSTP1*

As enzimas glutationas S-transferases (GSTs) possuem a função básica de detoxificação, mediando a conjugação de um grande número de compostos eletrofílicos com glutationa reduzida (GSH), numa reação metabólica de fase II (Pickett e Lu, 1989, Tijhuis et al., 2007). Estas enzimas possuem a capacidade de detoxificar os metabólitos reativos dos PAH, evitando que eles reajam com o DNA (Amorim *et al*., 2002), e também atuam na proteção contra produtos do estresse oxidativo (Hayes, 1995 citados por Egan *et al*., 2004). Além da detoxificação da fase II, as GSTs também desempenham papel importante na modulação da indução de outras enzimas e proteínas que atuam em outras funções celulares, como reparo de DNA (Mo *et al*, 2009).

As GSTs, em seres humanos, foram divididas em quatro classes alpha (α GSTA), mu (μ GSTM), pi (π GSTP) e theta (θ GSTT) (Mannervik , 1992). GSTs em humanos são polimórficos e um importante gene é *GSTP1*, que desempenha um papel fundamental na defesa do organismo contra vários tipos de câncer.

O *GSTP1* está localizado no cromossomo 11q18 (Yucesoy et al., 2012). Board *et al*. (1990) descreveram três diferentes alelos para a *GSTP1*: *GSTP1*A* (tipo selvagem), *GSTP1*B* e *GSTP1*C* (Board *et al*., 1990). A variação *GSTP1*B* apresenta uma transição de adenina para guanina na posição +313, códon 105, exon 5, o que resulta na substituição do aminoácido isoleucina para valina (Strange & Fryer, 1999, Rednam et al., 2012). Este polimorfismo está associada com um risco aumentado de desenvolvimento de câncer de bexiga, testículos e próstata [Harries et al., 1997], faringe, laringe e pulmão [Leichsenring et al., 2006]. A substituição da Isoleucina por

valina (códon 105) nos variantes *B* e *C* reduz a atividade catalítica da enzima (Pandya *et al*., 2000), que induz uma detoxificação menos eficiente de certos compostos mutagênicos e carcinogênicos, o que pode levar a aumento dos níveis de danos no DNA e consequente mutações e aumentar o risco de desenvolvimento de câncer (Ali-Osman *et al*., 1997, Canalle *et al*., 2004).

A GSTP1 metaboliza diversos compostos carcinogênicos, dentre eles o PAH, presentes em carnes excessivamente grelhadas e o benzo(alfa)pireno diolepóxido, que é um dos maiores metabólitos carcinogênicos derivados do tabaco (Hayes *et al*., 1995, Sugimura et al., 2004). A GSTP1 tem importância não somente na detoxificação de carcinógenos presentes em produtos industrializados, carnes grelhadas a altas temperaturas, como na detoxificação de carcinógenos inaláveis (Honma *et al*., 2008).

Um estudo realizado no Brasil associou os genótipos *Isoleucina/Valina* (genótipo *AG*) e *Valina/Valina* (genótipo *GG*) do *GSTP1* em combinação com polimorfismos dos genes *CYP1A1* e *CYP2E1* com risco aumentado de leucemia linfocitária aguda em crianças (Canalle *et al*., 2004). Um estudo com portadores de câncer colorretal não verificou associação desta doença com os genótipos *Isoleucina/Isoleucina* (genótipo *AA*), *Isoleucina/Valina* (genótipo *AG*) e *Valina/Valina* (genótipo *GG*), porém em combinação com os genótipos *GSTM1* nulo e *GSTT1* nulo, os portadores dos genótipos *GSTP1 AG* ou *GSTP1 GG* apresentaram risco aumentado de desenvolvimento de câncer colorretal (OR = 2.69, 95% IC: 1.02–7.11) (Ates *et al*., 2005). Por outro lado, a *GSTP1* não teve associação com risco de desenvolvimento de câncer de próstata (Mo *et al*., 2009). No Brasil este gene não teve associação com o risco de desenvolvimento de câncer de pulmão (Honma *et al*., 2008).

I.3.2 Metabolismo do Carbono

O metabolismo do carbono, no qual o ácido fólico desempenha um papel relevante é essencial para o metabolismo do DNA, pois é responsável pela regulação da expressão gênica, síntese de nucleotídeos e reparo de DNA (Fenech, 2001). O ácido fólico está relacionado a enzima metilenotetrahidrofolato redutase (MTHFR), que cataliza a conversão da 5,10-metilenotetrahidrofolato (5,10-THF) em 5-metilenotetrahidrofolato (5-THF), a forma predominante do ácido fólico circulante (Wiemels et al., 2001). Este, por sua vez, fornece grupos metil para a remetilação da homocisteína em metionina (Bailey *et al*., 2002; Chojnicka et al., 2012), que é um aminoácido essencial à síntese da S-adenosilmetionina (SAM), que é a principal doadora de grupos metil às reações de metilação, inclusive para a metilação do DNA (Selhub e Miller, 1992, Zingg e Jones, 1997), que atua na regulação da expressão gênica e na conformação da molécula de DNA (Duthie, 1999). Em condições de deficiência de acido fólico, a concentração de SAM é diminuída podendo levar a hipometilação do DNA (Duthie, 1999). Esta hipometilação pode acometer regiões do genoma como os protoncogenes, favorecendo o risco de desenvolvimento de câncer (Yi *et al*., 2000). Além disso, a deficiência do ácido fólico diminui a conversão de homocisteína em metionina causando um acúmulo de homocisteína no sangue, o que pode gerar espécies reativas de oxigênio (ROS), levando a danos oxidativos no DNA (Oikawa *et al*., 2003). Estudos demonstraram que o aumento da taxa de quebras cromossômicas está relacionado com a elevada concentração de homocisteína (Fenech *et al*., 1998; Trimmer et al., 2012). A deficiência do ácido fólico também leva a uma menor concentração de 5,10-THF, que é utilizada para a síntese de purinas além de fornecer grupos metil para a enzima timilidato sintetase (TS), que converte a deoxiuridina monofosfato (dUMP) em

deoxitimidina monofosfato (dTMP). Portanto, na deficiência de ácido fólico e consequentemente menor concentração de 5,10-THF, há menor síntese de purinas, e maior nível de incorporação de uracila ao DNA no lugar da timina (Eto e Krumdieck, 1986). Essa incorporação errônea da uracila pode ser tão intensa que as vias de reparo por excisão de base podem ser sobrecarregadas, levando a colapso do sistema reparo do DNA, com acúmulo de sítios apurínicos/apirimidínicos, quebras de fita simples e dupla o que pode levar a mutações pontuais e aberrações cromossômicas (Blount e Ames, 1995; Blount *et al*., 1997; Ames, 2001; Fenech, 2001).

Polirmorfismos de genes do metabolismo do ácido fólico em combinação com níveis de ácido fólico podem determinar as concentrações de SAM, homocisteína, 5,10-THF, 5-THF, e, portanto a estabilidade genômica.

I.3.2.1 Polimorfismos do gene *MTHFR*

O gene que codifica a enzima MTHFR foi mapeado no cromossomo 1, região 1p 36.3 e apresenta 11 éxons que variam de 102 a 432 pares de base em extensão (Pereira et al., 2006). O polimorfismo *C667T* do gene *MTHFR* resulta na substituição de uma alanina por uma valina (Frosst *et al*., 1995, Feng et al., 2009). Indivíduos heterozigotos (*CT*) e homozigotos (*TT*) para este polimorfismo possuem 30% e 65% da atividade enzimática normal *in vitro*, respectivamente (Frosst *et al*., 1995, Crott *et al*., 2001, Izmirli, 2013).

Indivíduos homozigotos *TT* estão associados com maior concentração de homocisteína no plasma, devido a baixa atividade enzimática (Kluijtmans *et al*., 1997, Zittoun *et al*., 1998, Trimmer et al., 2012). Por este motivo, este genótipo também está associado ao maior risco de desenvolvimento de doenças cardiovasculares devido ao

maior nível de homocisteína circulante (Sadeghian *et al*., 2006). Porém, diversos estudos associaram o genótipo *TT* combinado a adequada concentração de ácido fólico com menor risco de câncer colorretal (Ma *et al*., 1997, Chen *et al*., 1999) e leucemia linfocitária aguda (Skibola *et al*., 1999, Wiemels *et al*., 2001). Por outro lado, outros estudos associaram este genótipo ao maior risco de câncer endometrial (Esteller *et al*., 1997). Em condições de baixo consumo de ácido fólico, o genótipo *TT* do *MTHFR* foi associado ao maior risco de desenvolvimento de câncer gástrico (Miao *et al*., 2002). Os danos no DNA associados ao genótipo *TT* apresentam resultados contraditórios. Crott *et al* (2001) não encontraram efeito do polimorfismo *C677T* sobre danos cromossômicos *in vitro*. Devos et al (2008) verificaram que o genótipo *TT* diminuiu a incorporação de uracilas ao DNA em aproximadamente 34%. Narayanan *et al*. (2004) não encontraram influência dos polimorfismos do *MTHFR* sobre os níveis de danos no DNA.

O polimorfismo *A1298C* do *MTHFR* resulta na substituição do glutamato por uma alanina e está associado à redução de 60% da atividade enzimática em homozigotos *CC* (van der Put *et al*., 1998, Wisberg *et al*., 1998; Izmirli, 2013). Estudos indicam um menor risco de leucemia linfocitária aguda em adultos e crianças com os genótipos heterozigotos (*AC*) e homozigotos (*CC*) (Skibola *et al*., 1999, Wiemels *et al*., 2001). Outros estudos demonstraram uma associação inversa entre o genótipo *CC* e câncer colorretal (Chen *et al*., 2003; Keku *et al*., 2002; Murtaugh *et al*., 2007; Izmirli et al., 2013). Estudos associaram a combinação entre os polimorfismos *C677T* e *A1298C* com o risco de desenvolvimento de câncer, por exemplo: indivíduos portadores dos genótipos *1298AA/677TT* ou *1298CC/677CC* apresentaram redução de 2,2 e 3,3 vezes, respectivamente do risco de desenvolvimento de leucemia linfocitária aguda infantil (Krajinovic *et al*., 2004).

I.3.3 Sistema reparo de DNA

Vários estudos têm relatado a existência de grande variação inter-individual na capacidade de reparo do DNA (Vodicka *et al*., 2004; Liu et al., 2012). Indivíduos com menor capacidade de reparo de DNA apresentam maior risco de desenvolvimento de vários tipos de câncer (Vodicka *et al*., 2004). Polimorfismos de nucleotídeo único (SNPs) em seqüências codificadoras ou reguladoras podem levar a alterações nas enzimas de reparo de DNA, modulando a suscetibilidade ao câncer (Vodicka *et al*., 2004).

I.3.3.1 Polimorfismos do gene XRCC1

O gene de reparo *XRCC1* localizado no braço curto do cromossomo 19 (Mohrenweiser *et al.,* 1989, Ginsberg et al., 2011) codifica a proteína XRCC1 envolvida no sistema de reparo de excisão de bases (BER), que forma um complexo com outras enzimas envolvidas no reparo de quebras de fita simples do DNA por excisão de bases: DNA ligase III, DNA polymerase β e polyadenosina difosfato (ADP) ribose] polimerase (PARP) (Abdel-Rahman *et al*., 2000; Ginsberg et al., 2011; Przybylowska-Sygut et al., 2013). A proteína XRCC1 tem papel importante em diversas etapas do processo de reparo de quebras de fita simples do DNA, otimizando o reparo de danos no DNA causados por ROS, agentes ionizantes e alquilantes (Abdel-Rahman *et al*., 2000). O significado funcional destas variantes do gene *XRCC1* não foi ainda elucidado, mas alguns dos polimorfismos estão associados a uma capacidade reduzida de reparo, conseqüente acumulo de danos no DNA e aumento da susceptibilidade a condições

adversas de saúde, incluindo o câncer e a aterosclerose coronariana (Hung et al, 2005.; Bazo et al., 2011).

Já foram descritos e validados mais de sessenta polimorfismos do gene *XRCC1* (Hung et al., 2005), dos quais os mais relevantes ocorrem em três sequências conservadas e resultam em substituições de aminoácidos (Shen *et al*., 1998). Estes três polimorfismos foram detectados nos códons 194 (Arg→Gln), 280 (Arg→His) e 399 (Arg→Gln) (Ginsberg et al., 2011), numa freqüência alélica de 0,25, 0,08 e 0,25, respectivamente. Alguns estudos associaram estes polimorfismos a diferentes riscos de desenvolvimento de câncer e níveis de danos no DNA (Vodicka *et al*., 2004). Um estudo mostrou que indivíduos com o alelo *399gln* tiveram um aumento do risco de danos cromossômicos, enquanto que indivíduos com o alelo 1*94trp* tiveram uma redução desses riscos (Skjelbred *et al*., 2006[a]). Os mesmos autores demonstraram aumento do risco de desenvolvimento câncer colorretal em indivíduos com o polimorfismo *280Hist* e menor risco em indivíduos com polimorfismo *399Gln* (Skjelbred *et al*., 2006[b]). Outro estudo associou o alelo *399Gln* a maior frequência de micronúcleos em indivíduos fumantes (Mateuca *et al*., 2008). Li *et al* (2009) avaliaram a associação dos polimorfismos do *XRCC1* com a evolução de lesões gástricas pré-cancerosas associadas à *H. pylori*, e verificaram que indivíduos portadores do genótipo *194Arg/Trp + 194Trp/Trp* apresentaram maior chance de regressão das lesões gástricas, enquanto que indivíduos com o genótipo *399Arg/Gln + 399Gln/Gln* tiveram uma diminuição da chance de regressão das lesões (Li *et al*., 2009). Em estudo de meta-análise Kiyohara *et al* (2006) demonstraram associação do genótipo *399Gln/Gln* (genótipo *GG*) com maior risco de desenvolvimento de câncer de pulmão em asiáticos (Kiyohara *et al*., 2006).

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III – OBJETIVOS

Devido à importância dos fatores alimentares e genéticos no processo das doenças degenerativas, o presente projeto tem os seguintes objetivos:

1. Quantificar os níveis de danos oxidativos no DNA e incorporação de uracila no DNA e a eficiência do sistema reparo do DNA em linfócitos de sangue periférico de indivíduos com diferentes padrões alimentares.
2. Verificar a influência dos níveis de luteína, criptoxantina, α-caroteno, β-caroteno, licopeno, retinol e α-tocoferol plasmáticos com danos oxidativos no DNA e incorporação de uracila no DNA e com a eficiência do sistema reparo do DNA em linfócitos de sangue periférico de indivíduos com diferentes padrões alimentares, sub-agrupados de acordo com os percentis de concentrações plasmáticos de cada micronutrientes.
3. Verificar a influência dos polimorfismos dos genes *XRCC1* (nos *codons 194* e *399,* rs1799782 e rs25487, respectivamente), *MTHFR (C677T* e *A1298C,* rs1801133 e rs1801131, respectivamente)*, CYP1A1* (rs1048943*)* e *GSTP1* (rs1695) sobre os níveis de danos oxidativos no DNA, incorporação de uracila no DNA e a eficiência do sistema reparo do DNA em linfócitos de sangue periférico de indivíduos com diferentes padrões alimentares.

Manuscrito I

Trabalho elaborado segundo as normas da revista “The Journal of Nutrition”.

Can diet rich in whole grains, fruits and vegetables and low in processed products associated with a healthy lifestyle protect against oxidative DNA damage?

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Running title: Associations between micronutrients and DNA damage

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Abstract

The present study evaluated the influence of diet on the level of oxidative DNA damage, incorporated uracil and DNA-repair capability in peripheral blood lymphocytes of two groups of individuals with antagonist diets: 1) 87 healthy individuals with a diet rich in organic products, whole grains, fruit and vegetables and poor in industrialized products (Group I); 2) 97 healthy individuals with diet rich in industrialized products and poor in fruit and vegetables (Group II), besides we evaluated the influence of α- and β-carotenes, lycopene, lutein, criptoxanthin, retinol (carotenoids) and α-tocopherol (present in their diet and not supplementing) on strand breaks (SBs), oxidative DNA damage, misincorporated uracil and DNA repair capability. SBs, oxidative DNA damage, misincorporation uracil and DNA repair capability were assessed by the comet assay. The levels of carotenoids and α-tocopherol in the plasma were measured using high-performance liquid chromatography (HPLC). The individuals in Group I presented lower levels of oxidative DNA damage and lower levels of DNA damage induced by H_2O_2 than individuals of Group II. Regarding the subgroups of micronutrients: Individuals of the subgroups 75th percentile for all micronutrients had a higher level of DNA damage than individuals of the 50th percentile and higher level of DNA damage than individuals of the subgroup 25th percentile only relation to β- carotene, retinol and α- tocopherol, Moroever, the subgroups 25th percentile of lutein, cryptoxanthin and α-carotene had a higher level of DNA damage than individuals at 50th percentile. Individuals of the subgroups 25^{th} percentile for all micronutrients had a higher level of oxidized purines and higer levels of oxidized pyrimidines to lutein than individuals of the 50th percentile. Moreover, individuals of the 25^{th} percentile of lutein, α- carotene and β- carotene had a higher level of oxidized purines and oxidized pyrimidines (only for lutein) in relation to individuals of the 75^{th} percentile. Individuals of the subgroups

75^{th} percentile of lutein, lycopene, α- carotene, β- carotene, retinol and α- tocopherol had a higher level of uracil misincorporated in DNA than individuals of the 50th percentile and a higher level of uracil misincorporated than individuals of the 25th percentile to retinol. Individuals of the 75th percentile of α- tocopherol and lutein had a higher level of DNA damage induced by H_2O_2 and lower level of DNA repair capability than individuals of the 50th percentile, respectively. The analysis of our results provide evidence that a diet rich in whole grains, fruit and vegetables and poor in industrialized products can protect against oxidative DNA damage. Nevertheless, it is important to have an appropriate intake of nutrients, because both deficiency and excess of micronutrients can lead to increase of DNA damage.

1. Introduction

Diet certainly plays an important role in chronic degenerative diseases as cancer, diabetes mellitus II, Alhzeimer, Parkinson, infertility, immune dysfunction, and cardiovascular diseases [1; 2; 3; 4]. However, the mechanism is still not clear [5]. It is increasingly evident that nutrigenetics and nutrigenomics has a central role in the investigation of the effect of nutrition on health outcomes, and impacts of nutrients that can be thoroughly evaluated by a multitude of technologies 'omics' and biomarkers [6]. Among these biomarkers, only DNA damage is a clear biomarker of fundamental pathology that may be mitigated by promotion of apoptosis of genetically aberrant cells or by reducing the rate of DNA damage accumulation [6]. Thus, the significance of DNA damage as a precursor of chronic degenerative diseases is clear [7].

Several in vitro studies has been demonstrated that some micronutrients present in the diet (carotenoids and vitamins) have an antioxidant potential, that show a cancer protective effect, since they act as a first line of cellular defense against DNA oxidation [7]. They act as antioxidants to be efficient scavengers of various free radicals generated *in vitro* [8; 9] and they can also act as cofactors enzymatic or as part of the structure of proteins (metal-loenzymes) involved in DNA synthesis and repair, prevention of oxidative damage to DNA, and maintenance methylation of DNA and telomeres [10; 11]. Recent studies are also describing that carotenoids may mediate their effects via other mechanisms such as gap junction communication, tumor-supressive activity, carcinogenesis, protection of DNA cell growth regulation, immune response, protect lipid against peroxidation, and as modulators of Phase I and II drug metabolizing enzymes modulating gene expression [12; 13 ; 14; 15]. However, the supplementation of carotenoids is still unclear and controversy. For example, results from interventions

studies involving β-carotene supplementation were conflicting, with no effect, decrease and increase of incidence of cancer [16]. Therefore, the evidence of a cancer protective action of carotenoids in humans is, to say the least, a mistake [9].

This occured maybe due to the imbalaced administration of supplement or high dietary intake. Both micronutrient deficiency and micronutrient excess can cause genome damage that is equivalent to genome damage levels caused by exposure to significant doses of environmental genotoxins, such as chemical carcinogens and ultra-violet and ionizing radiation [10]. Some recent studies have shown that carotenoids in high concentrations may cause prooxidant effects [17]. For example, experiments with β-carotene alone have shown increases in DNA strand breaks (SBs) [18], DNA fragmentation [19], and oxidised purines [20; 21]. In addition, in an other study, β-carotene supplemented smokers were found to have increased lung cancer mortality when compared to the group not receiving the β-carotene supplement [22]. So, it is fundamental, in these areas, characterize ideal dietary intakes for preventing DNA damage and aberrant gene expression for genetic sub-groups and for each individual, since the amount of micronutrients that appear to be protective against genome damage varies greatly among food types, and a careful choice is needed to design dietary patterns or dietary reference values optimized for genome health maintenance [11].

Epidemiological studies have shown the importance of a diet rich in fruits and vegetables in the prevention of illnesses, and low or very high plasma concentrations of micronutrients with antioxidant properties, found in fruits and vegetables, are associated with increased risk for some diseases, as decribed above. So, based on these studies and evidences, this study aimed to evaluate the influence of diet on the level of oxidative DNA damage, incorporated uracil and DNA-repair capability in peripheral blood lymphocytes of two groups of individuals with antagonist diets, besides to match the

level of α- and β-carotenes, lycopene, lutein, criptoxanthin, retinol and α-tocopherol (present in their diet and not supplementing) on the oxidative DNA damage, misincorporated uracil and DNA repair capability in subgroups of healthly brazilian volunteers with different micronutrients frequency distribution quantified in their plasma.

2. Material and Methods

The present study was developed in Botucatu Medical School-UNESP, departament of Pathology, in Botucatu, State of São Paulo, Brazil and Tufts University, Jean Mayer USDA-HNRCA, Boston, MA, USA. It was approved by the local Ethical Committee.

Volunteers

A total of 202 healthy adult volunteers were interviewed. From these volunteers, a group of 18 individuals were excluded from the study due to technical problems or because did not fit in the parameters established. Therefore, 184 healthy adult volunteers (average age 32,5 ± 11,47) 72 men and 112 women, were evaluated in this study, which is a continuation of a previous study [23]. All of the volunteer were nonsmokers, were not abusing alcohol, and were not using supplementation, prescription or recreational drugs. In all volunteers recruited were measured the level of SBs, oxidative DNA damage, uracil misincorporation and DNA repair capability. However, we analyzed the levels of carotenoids, retinol and α-tocopherol just in 172 of these volunteers.

All participants donated a blood sample and an informed consent to participate was obtained from all the volunteers. From each volunteer was collected, by trained personnel and using disposable material, 20 ml of peripheral blood. Of those, 2 ml were used to detect SBs, oxidative DNA damage, uracil misincorporation and DNA repair capability, which was measured by using the comet, 4 ml for extraction of DNA and RNA for determination of polymorphisms and gene expression profile, respectively (future studies), and 14 ml for micronutrients analysis, which was evaluated by high-performance liquid chromatography (HPLC).

The volunteers were divided in two groups for analysis of genome damage in lymphocytes: 1) 87 naturalistic individuals with a uncommon life style characterized by absence of urban stress, bucolic life and great consume of organic fruits, vegetables and juice and poor consume of industrialized foods (Group I); 2) 97 individuals with great ingestion of industrialized foods and poor ingestion of fruits and vegetables (Group II).

For evaluation of the dietary pattern a Food-frequency Questionnaire adapted from Cardoso and Stocco [24] was applied, and was used for the classification of the groups. The referred questionnaire is quantitative and has provided data, which were inserted in the program AvaNutri (Avanutri Informática Ltda). This procedure enabled us to establish an estimate of the quantity of protective foods consumed daily by the population studied. Individuals classified in Group I, had to consume daily the percentile 75 of organic fruits and vegetables and below the percentile 25 of each industrialized products. The individuals of group II were those who consumed less than percentile 25 of organic fruits and vegetables and above the percentile 75 of industrialized products.

Furthermore, to investigate the effects of each micronutrient on DNA damage, we regrouped these individuals into subgroups according to percentiles of each

micronutrients frequency distribution quantified in plasma of these volunteers. The effect of each micronutrient on genome stability is described as the percent variation of the mean DNA damage in the subgroups: 1) 25^{th} percentile (lowest levels of micronutrient), 2) 50^{th} percentile (micronutrient levels between the 25th and 75th percentiles/ median levels) and 75^{th} percentile (higher levels of micronutrients) of micronutrients frequency distribution. Therefore, we have created three subgroups for each micronutrient.

Only were selected individuals that have adopted the respective diet pattern for at least 10 years.

Determination of SBs, Oxidative DNA Damage and level of uracil incorporate into DNA

SBS, oxidative DNA damage, uracil misincorporation into DNA, and DNA repair were evaluated as described and accomplished by Prado et al in a previous study [23]. The alkaline Comet assay [25], modified with lesion-specific enzymes was used to detect single and double strand breaks, labile sites (SBs), oxidised purines and pyrimidines and uracil [26]. All procedures were conducted in the dark to minimize spurious sources of DNA damage. Briefly, 10 μl of the isolated lymphocytes suspension [27] (≅ 2 x 10^4 cells) were embedded into 0.5% low melting point agarose (Sigma) and spread on agarose-precoated microscope slides. Twelve slides were prepared for each sample. Slides were immersed overnight in freshly prepared cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium salt N-Lauryl sarcosine, pH 10.0, with 1% Triton X-100 and 10% DMSO added fresh) at 4^0C. After lysing, the slides were washed three times in enzyme buffer (Trevigen, Gaithersburg, MD) and then three

slides for each treatment were incubated at 37°C for 45 min with 100 μL of endonuclaese III (Endo III - 1:1000; New England Biolabs Inc), 100 μL of formamidopyrimidine-DNA glycosylase (FPG - 1:1000; New England Biolabs Inc), 100 μL of uracil-DNA glycosylase (UDG - 1:1000; New England Biolabs Inc) or with enzyme buffer only. Endo III recognizes oxidised pyrimidines (SBs EndoIII), while FPG identifies oxidised purines (SBs FPG), especially 8-oxo-guanine and UDG recognizes uracil (SBs UDG). Enzyme buffer only is used to identified SBs. Subsequently the cells were exposed to alkali buffer (1mM EDTA and 300 mM NaOH, pH ≅ 13.4), at 4°C, for 40 min to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was conducted in the same solution at 4°C, for 30 min, at 25 V (1v/cm) and 300 mA. After electrophoresis, the slides were neutralized (0.4 M Tris, Ph 7.5), stained with 50 μL Syber Green (1:10000; Invitrogen), and analyzed in a fluorescence microscope at 400 x magnification, using an image analysis system (Comet Assay II – Perceptive Instruments, Suffolk, UK).

For each individual, one hundred randomly selected cells (50 from each of two replicate slides) for each treatment (FPG, Endo III, UDG or enzyme buffer only) were evaluated and the mean tail intensity (%DNA Tail) was determined, totaling 400 cells analyzed from each individual. Tail Intensity, according to Comet Assay II – Perceptive instruments, is defined as "the sum of all intensity values in the tail region less those which are part of the mirrored head region. The value is also expressed as a percentage of the total Comet or cell intensity".

To analyze the influence of dietary patterns on the DNA repair capability, 200 μl of the freshly isolated lymphocytes were treated with 100 μl of H_2O_2 (100 μM), for 30 minutes, in ice, and analysis of the level of damage was evaluated. Then, after two wash

in PBS to remove of H_2O_2, these aliquots were incubated at 37 °C for 30 minutes, and DNA damage was checked again.

Carotenoids, retinol and α-tocopherol analyses

Volunteers provided blood samples on the day of the baseline interview. Blood samples were centrifuged at 2500 rpm for 30 minutes and plasma was separated and frozen at -80°C until analyzed for carotenoids, retinol and α-tocopherol. The micronutrients were measured using high-performance liquid chromatography (HPLC) as described by Yeum et al [28]. Briefly, 3 ml of CHCl3:MeOH were added into 100 µl of plasma in tubes, then 100 µl Echinenone diluted in ethanol (100 µL) was added as an internal standard, and 500 µl saline 0.85% was added and the mixture was shaken and centrifuged at 3000 rpm at 4°C for 10 minutes. The down layer were collected and separated in another tubes. In the tube which contain the other parts, 3 ml hexane was added and the tube was shaken and centrifuged at 3000 rpm at 4° C for 10 minutes again. The upper layer was removed and added to the separated tubes. The hexane layer was completely evaporated under N_2, and the residue was redissolved in 100 µL of ethanol, vortexed, and sonicated for 30 sec and transfer into a vial that was used for HPLC analysis.

The HPLC system was a Waters Alliance 2695 (Waters, Wilmington, MA, USA) and consisted of pump and chromatography bound to a 2996 programmable photodiode array detector, a C30 carotenoid column (3 mm, 150 3 4.6 mm, YMC, Wilmington, MA), and Empower software. The Waters 2996 programmable photodiode array detector was set at 340 nm for retinoids and 450 nm for carotenoids. Using this method, lutein, cryptoxanthin, β-carotene, α-caronete, lycopene, retinol and tocopherol

were adequately separated. Carotenoids and retinoids were quantified by determining peak areas in the HPLC chromatograms calibrated against known amounts of standards. The amounts were corrected for extraction and handling losses by monitoring the recovery of the internal standards. All the steps before were done in the red lamp.

Statistic Analyses

The data obtained in the Comet Assay had asymmetric distribution, then a nonparametric analysis was applied. The statistical analysis consisted of applying the Kruskal-Wallis Test to compare differences between the groups. A univariate procedure was applied to define the quartiles of each micronutrient. And an ad hoc multivariate model was performed for evaluated the effects of each micronutrient on the level biomarkers of DNA damage.

3. Results

DNA damage, as single- and double-strand breaks and apurinic/apyrimidinic sites (SBs), can be evaluated by the comet formed under standard conditions. Endogenous formation of oxidized pyrimidines and purines were detected by enzymes endonuclease III (EndoIII) and formamidopyrimidine DNA glycosylase (FPG), respectively. Even though in this study we have increased the sample size, the results remained in accordance with our previous study Prado et al., [23], which no significant differences in the levels of SBs and misincorporated uracil in DNA between the two groups were observed, but the volunteers in Group I presented a lower level of oxidized purines and pyrimidines ($p < 0.05$) than individuals in Group II (Figure 1). In addition, there was no impairment of DNA repair capacity in both groups, although the

individuals in Group I presented lower levels of DNA damage induced by H_2O_2 (Figure 2).

The means of 25th, 50th and 75th percentiles for the division of the subgroups for each micronutrient were respectively: for lutein 7.87 µg/dL, 16.06 µg/dL and 86.99 µg/dL; for cryptoxanthin 2.42 µg/dL, 10.26 µg/dL and 41.89 µg/dL; for lycopene 12.59 µg/dL, 23.42 µg/dL and 34.35 µg/dL, for α- carotene 2.98 µg/dL, 8.36 µg/dL and 17.64 µg/dL; for β- carotene 3.61 µg/dL, 15.70 µg/dL and 27.41 µg/dL; for retinol 41.97 µg/dL, 58.01 µg/dL, and 74.13 µg/dL; and for α- tocopherol 565.46 µg/dL and 1005.57 µg/dL 1291.38 µg/dL.

Then, we evaluated the influence of percentiles of micronutrients (25th, 50th and 75th) separately to each biomarker to DNA damage. Figure 3 shows the influence of the different micronutrients in the level of DNA damage under alkaline standard conditions (TI SBs). We observed that individuals of the subgroups 75th percentile for all micronutrients analyzed had a higher level of TI SBs than individuals of the 50th percentile ($p < 0.01$). Regarding β- carotene, retinol and α- tocopherol, individuals of the 75th percentile had also a higher level of TI SBs than individuals of the subgroup 25th percentile ($p < 0.01$). Moroever, the subgroups 25th percentile of lutein, cryptoxanthin and α- carotene had a higher level of DNA damage than individuals at 50th percentile ($p < 0.01$).

In relation to oxidative damage, we observed that individuals of the subgroups who presented the lowest levels of all micronutrients (25th percentile) had a higher level of oxidized purines (TI SBs FPG) than individuals of the 50th percentile (Figure 4). The same results were found to lutein regarding to oxidized pyrimidines (TI SBs EndoIII) (Figure 5). Moreover, individuals with levels of lutein, α- carotene and β- carotene in the 25th percentile had a higher level of oxidized purines (Figure 4) and oxidized

pyrimidines (only for lutein, Figure 5) in relation to individuals of the 75th percentile ($p < 0.01$).

The Figure 6 shows the influence of micronutrients levels in the level of uracil misincorporation on DNA (TI SBs UDG). We observed that individuals of the subgroups with highest level (75th percentile) of lutein, lycopene, α- carotene, β-carotene, retinol and α- tocopherol had a higher level of misincorporated uracil in DNA than individuals of the 50th percentile. In addition, the same individuals had a higher level of TI UDG than individuals of the 25th percentile to retinol. No significant differences to this biomarker between the subgroups 25th and 50th percentiles for all micronutrients were observed.

Regarding the effects of the micronutrients on the level of DNA damage induced by H_2O_2 and DNA repair capability (Figures 7 and 8, respectively), individuals of the 75th percentile of α- tocopherol and lutein had a higher level of DNA damage induced by H_2O_2 (TI SBs H_2O_2) and lower level of DNA repair capability (TI SBs H_2O_2R) than individuals of the 50th percentile, respectively. However, no significant differences in the levels of these biomarkers between the anothers subgroups were found.

4. Discussion

The field of nutrigenomics takes advantage of multiple disciplines and includes dietary effects on genome stability (DNA damage, chromosomal and molecular level), epigenome changes (DNA methylation), RNA and micro-RNA expression (transcriptomics), protein changes expression (proteomics) and metabolite (metabolomics), all of which can be studied independently or integrated to diagnose the health condition and / or disease-history trajectory [6]. However, among these biomarkers, only DNA damage is a fundamental biomarker to the disorders which can

be alleviated by promoting apoptosis of cells genetically abnormal or by reducing the rate of accumulation of DNA damage [6]. The Comet assay is technique widely used for detecting genotoxicity, which could lead to genomic instability. This assay has several advantages, as it has shown to be economical, simple, fast and requires small number of cells [29] and provides information on antioxidant status [30] and for this reason we chose this technique to ensure the correct analysis of the genotoxic effects induced by dietary factors, we adopted amendments to the modified protocol described by Singh et al. [31], which also allowed the detection of specific types of DNA damage, such as oxidative damage and uracil misincorporation besides SBs induced after exposure of intact cells to reactive oxygen species (ROS) [29]. In the present study, our main focus was on some carotenoids, retinol e α-tocopherol and their influence on SBs, oxidative DNA damage, misincorporated uracil and DNA repair capability in subgroups of healthly brazilian volunteers with different micronutrients frequency distribution quantified in their plasma, besides to evaluate the influence of diet on the level of oxidative DNA damage, incorporated uracil and DNA-repair capability in peripheral blood lymphocytes of two groups of individuals with antagonist diets.

Several epidemiologic studies have shown an association between foods intake rich in carotenoids and low incidence of various chronic degenerative diseases, such as various types of cancer and cardiovascular diseases [9; 13; 32], since oxidation levels of DNA damage, lipids and proteins induced by reactive oxygen species may be controlled by antioxidants, which are present in the diet [33]. We observed, in the present study, that the healthy individuals in Group I presented lower levels of oxidized purines and pyrimidines in peripheral-blood lymphocytes. Furthermore, a lower level of DNA damage was detected when the lymphocytes from these individuals were treated with H_2O_2 *ex vivo*. Perhaps, these lower levels of DNA damage could be attributed to the

micronutrients, vitamin C, vitamin E, carotenoids and flavonoids with antioxidant properties, present in the plants and fruit consumed by individuals in Group I (data not showed). These micronutrients can act as antioxidant, quenching singlet O_2 and inhibiting others free radicals or can act as enzymatic cofactors, modulating several biological processes that are relevant for chronic degenerative diseases, including xenobiotic metabolism, oxidative damage to macro-molecules, DNA repair and DNA methylation and apoptosis [34; 35]. On the other hand, the higher level of oxidative DNA damage detected in individuals in Group II could be related to consume of chemical additives, potentially genotoxics and carcinogenics [29], present in industrialized foods that constituted their diet. Several chemical food additives have proven to be carcinogenic, and many of these can react with DNA [36]. In addition, it is known that high ingestion of calories or certain foods, such as red meat or coffee, may cause adverse effects, for example, an increase in the DNA damage induced by heterocyclic aromatic amines [37].

However, reports in the literature involving carotenoids have been controversy. For example, β-carotene supplementation has been associated both positively and negatively with cancer incidence, but in some studies no effect has been reported [38; 39; 40; 41; 42]. According to Fenech [10], a limitation of some of these studies is that or they are generally accomplished during short periods of time, or limited to tissues, usually blood cells and single assays of DNA damage, differently from the present study that evaluated six types of biomarkers for DNA damage and only recruited individuals that have been adopted the respective diet pattern for at least 10 years. Furthermore, the quantities of micronutrients which appear to be protective against genome damage vary widely among foods [11], and careful selection is necessary to design dietary patterns optimized for genome health maintenance [10]. We found, in

this study, that the lowest percentile of levels of lutein, criptoxanthin, lycopene, α- and β- carotene, retinol and α-tocopherol was associated with significant increases in oxidative purines when compared to median levels of theses micronutrients. The same results were found to lutein regarding to oxidized pyrimidines. Moreover, individuals with levels of lutein, α- carotene and β- carotene in the 25th percentile had a higher level of oxidized purines and oxidized pyrimidines (only for lutein,) in relation to individuals of the 75th percentile. And the subgroups 25th percentile of lutein, cryptoxanthin and α-carotene had a higher level of DNA damage under alkaline standard conditions than individuals at 50th percentile. Our results are in agreement with the results reported by Bull and Fenech [11] that reported that genome damage caused by moderate micronutrient deficiency is of the same order of magnitude as the genome-damage levels caused by exposure to sizeable doses of environmental genotoxins such as chemical carcinogens, UV radiation and ionising radiation. This may be the reason why in low concentrations of antioxidant, free radicals can damage cellular proteins and lipids and form DNA adducts contributing to carcinogenic [43].

Nevertheless, several studies have shown that both micronutrient deficiencies and the excess can increase risk of carcinogenesis [34; 44, 45] and highlights the urgent need for better understanding of the dose-response relationship between micronutrient intake and genome damage [10]. Our data showed that: 1) the highest percentile of levels of all micronutrients was associated with significant increases in DNA damage when compared to the median levels and 2) the highest percentile of levels of β-carotene, retinol and α- tocopherol was associated with significant increases in DNA damage relative to the lowest percentile. Furthermore, individuals of the 75th percentile of lutein had a lower level of DNA repair capability (TI SBs H_2O_2R) than individuals of the 50th percentile. Based on our data, it is important to note that excess of

micronutrient can be so deleterious as deficiency, mainly in the subgroup of individuals with 75th percentile of α- tocopherol that had a higher level of DNA damage induced by H_2O_2, in another words, the wide availability of micronutrients with antioxidant properties did not show a protective effect when the offer of free radicals have been larger. These results are consistent with data in the literature that suggest an increased cancer risk with deficiency or supplementation above the Recommended Dietary Intake for this vitamin [44; 46]. Moreover, when some carotenoids intake is higher, they may act as pro-oxidant, causing toxic effect, such as increased single or double breaks strand and inhibiting the expression of enzymes glutathione S-transferase (GSTs) whose enzyme activity can be beneficial to cancer prevention [47]. Our results are in agreement with the results reported by Fenech [10] that reported that the highest percentile of intake of β- carotene, riboflavin, pantothenic acid, and biotin was associated with significant increases in micronuclei frequency. Therefore, micronutrient deficiency and vitamin excess can increase DNA damage and risk of development of chronic degenerative diseases as cancer, that is supported by several studies [10], and help to explain why our results not show significant difference between the subgroups 75^{th} and 25^{th} of lutein, criptoxanthin, lycopene and α-carotene in relation to DNA damage, between the subgroups 75^{th} and 25^{th} of lycopene, retinol and α-tocopherol in relation to oxidized purines, between the subgroups 75^{th} and 25^{th} of criptoxanthin, lycopene, α- and β- carotene, retinol and α-tocopherol in relation to oxidized pyrimidines and between the subgroups 75^{th} and 25^{th} of lutein, criptoxanthin, lycopene, α- and β- carotene and and α-tocopherol in relation to uracil misincorporated in DNA. Recommends that the ingestion of carotenoids does not exceed 10 mg/day, especially in the case of smokers [48].

Several micronutrients act as cofactors enzymatic in DNA replication, DNA stability, DNA repair, and DNA methylation and apoptosis [34] and modulate expression of certain genes [9]. For example, the active carotenoids increase the expression of the connexin 43 gene [49] and α-carotene suppresses synthesis of N-mycRNA, arresting neu-roblastoma cells in G0 [9;50]. We observed that individuals of the subgroups with highest level (75^{th} percentile) of cryptoxanthin, lycopene, α-carotene, β- carotene, retinol and α- tocopherol had a higher level of uracil misincorporated in DNA than individuals of the 50th percentile. In addition, the same individuals had a higher level of uracil misincorporated than individuals of the 25th percentile to retinol. Perhaps, these micronutrients in excess might inhibit the expression of genes related to carbon metabolism that could lead to uracil misincorporation, that is a type of damage that can occur either during DNA replication as a result of uracil incorporation instead of thymidine, or is formed from the endogenous hydrolytic deamination of cytosine [51], being probably the major cause of endogenous DNA damage [52].

Although freshly isolated lymphocytes appear to be relatively deficient in the repair of H_2O_2-induced damage; even after several hours, DNA breaks have not fallen to the basal level [9;53], we only have observed that individuals of 75^{th} percentile of lutein had a higher level of DNA damage than individuals of the 50th percentile, regarding a DNA repair capability. However, we did not found an impairment of DNA repair capability in other subgroups. These DNA lesions are repaired by different pathways, which could affect the results. This may be the reason why no difference was found between the subgroups as regards DNA repair efficiency.

Several studies have been reported the difficult to determine the real effect of nutrients isolated in chemoprevention cancer [54]. Since that the micronutrients could

interact and lead to synergistc effect, as for example the interaction between the compounds poliphenolics and fiber diet that decreased the risk of colorectal cancers [55]. The combination of β-carotene with ascorbic acid and α-tocopherol increases to protective effective, since such nutrients may interact in cellular environment and to potentiate the antioxidant defense [56] resulting, for example, in protection against oxidative damage in the DNA [57]. Perhaps, this is the reason why we found no significant difference between the subgroups 75^{th} and 50^{th} of all micronutrients in relation to oxidized purines and pyrimidines, or between the subgroups 75^{th} and 50^{th} of criptoxanthin in relation to uracil misincorporated, or between the subgroups 75^{th} and 50^{th} of lutein, criptoxanthin, α and β- carotene, lycopene and retinol in relation to DNA damage induced by H_2O_2. However, even if the precise intake levels were known, the biological 'dose' will vary greatly between individuals because of genetic variability affecting either the absorption, biotransformation, metabolism, distribution or elimination of a nutrient or food bioactive and cellular mechanisms of action, such as binding to nuclear receptors or regulating transcription factors [58].

In conclusion, our results provide evidence that a diet rich in whole grains, fruit and vegetables and poor in industrialized products associated with a healthy lifestyle can protect against oxidative DNA damage. These findings help to explain why fruits and vegetables are often linked to lower risks in the development of some diseases, such as cancer, eye disease and cardiovascular disease (CVD). Nevertheless, it is important to have an appropriate intake of nutrients, because both deficiency and excess of micronutrients can lead to DNA damage. Further studies on a larger number of individuals must be conducted in order to also evaluate the influence of gene polymorphisms.

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Legends

Figure 1. Levels of DNA damage (tail intensity) expressed as TI SBs, TI SBs FPG, TI SBs Endo III, TI SBs UDG, TI SBs H_2O_2 and TI SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=90) and Group II (n=95) individuals. TI SBs = single and double strand breaks and alkali labile sites; TI SBs FPG = SBs more FPG sensitive sites (purine oxidized); TI SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized) and TI SBs UDG = SBs more misincorporated uracil sites. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5^{th} and 95^{th} percentiles at the whiskers. The Kruskal-Wallis Test was used to compare differences between groups. * $p < 0.05$. The individuals in Group I presented lower levels of oxidized purines (SBs FPG) and pyrimidines (SBs Endo III) than did individuals in Group II.

Figure 2. Levels of DNA damage (Tail Intensity) expressed as TI SBs H_2O_2 and TI SBs H_2O_2R in peripheral blood lymphocytes of individuals in Group I (n = 90) and Group II (n=95); H_2O_2 = strand breaks induced by treatment with H_2O_2; H_2O_2R = level of DNA damage 30 minutes after DNA repair of damage induced by treatment with H_2O_2. Results are expressed as mean ± SD. The Kruskal-Wallis Test was used to compare differences between variables.* $p < 0.05$. Individuals in both groups did not present inhibited DNA repair. Individuals in Group I presented lower levels of DNA damage induced by H_2O_2.

Figure 3. Influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β-carotene, retinol and α- tocopherol on the level of DNA damage under standard conditions (TI SBs). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters mean no

significant difference between subgroups. Different letters mean that there were differences on the level of DNA damage under standard conditions between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Figure 4 - Represents influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β- carotene, retinol and α- tocopherol on the level of oxidized purines (TI SBs FPG). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters mean no significant difference between subgroups. Different letters mean that there were differences on the level of oxidized purines between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Figure 5 - Represents influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β- carotene, retinol and α- tocopherol on the level of oxidized pyrimidines (TI SBs EndoIII). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters or without letters mean no significant difference between subgroups. Different letters mean that there were differences on the level of oxidized pyrimidines between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Figure 6 - Represents the influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β- carotene, retinol and α- tocopherol on the level of misincorporated uracil in DNA (TI SBs UDG). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters or without letters mean no significant difference between subgroups. Different letters mean that

there were differences on the level of misincorporated uracil in DNA between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Figure 7 - Represents the influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β- carotene, retinol and α- tocopherol on the level of DNA damage induced by H_2O_2 (TI SBs H_2O_2). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters or without letters mean no significant difference between subgroups. Different letters mean that there were differences on the level of DNA damage induced by H_2O_2 between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Figure 8 - Represents the influence of the subgroups of lutein, criptoxanthin, lycopene, α – carotene, β- carotene, retinol and α- tocopherol on the level of DNA repair capability (TI SBs H_2O_2R). Results are expressed as percentiles bars that correspond the subgroups 75^{th}, 50^{th} and 25^{th} percentiles of micronutrients. Same letters or without letters mean no significant difference between subgroups. Different letters mean that there were differences on the level of DNA repair capability between subgroups. The multivariate model was used to compare the differences between groups. * P <0.05.

Influence of *dietary pattern* on DNA damage (Tail Intensity).

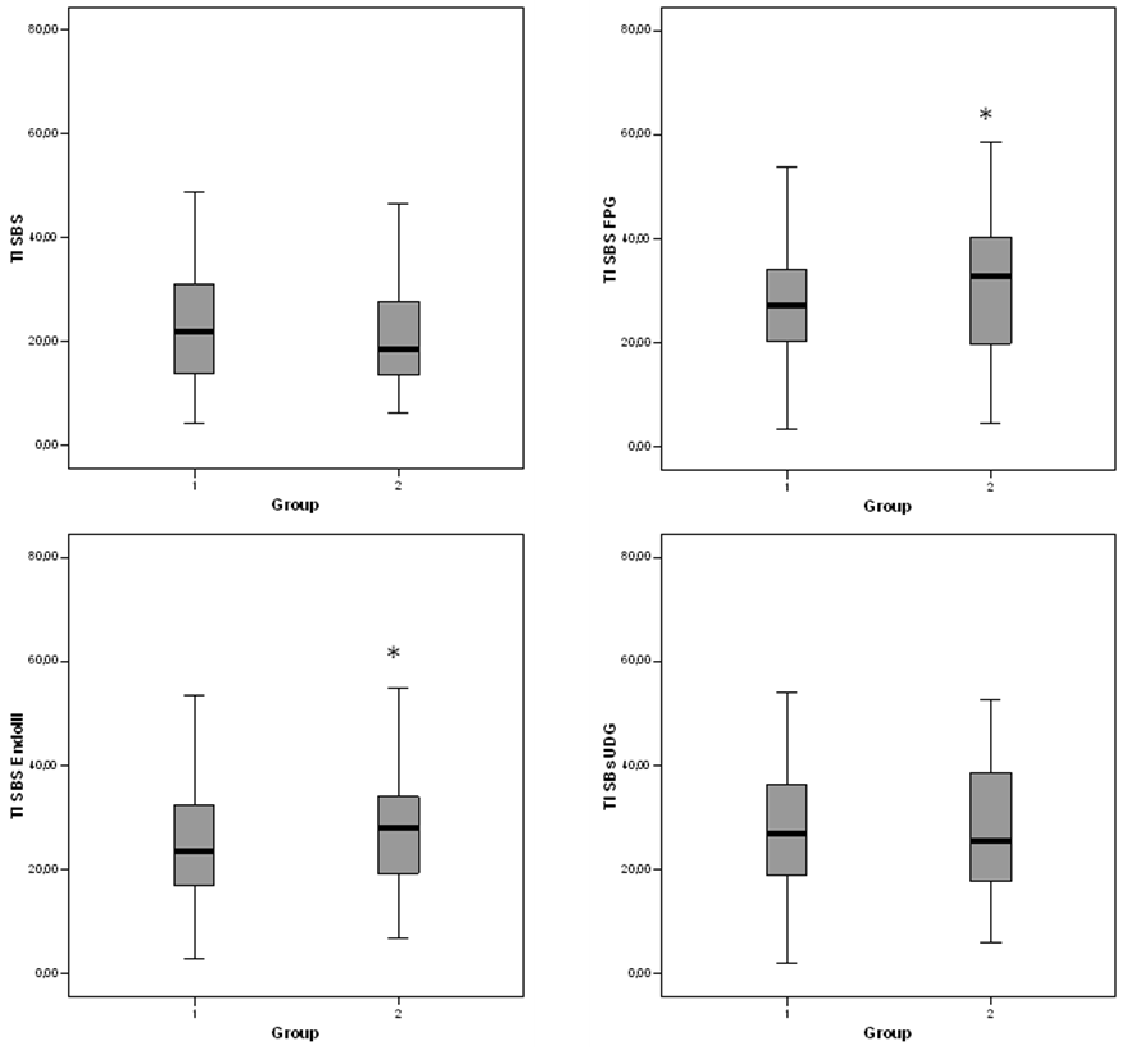

Figure 1

Influence of *dietary pattern* on DNA damage induced by H2O2 and DNA Repair Capability (Tail Intensity).

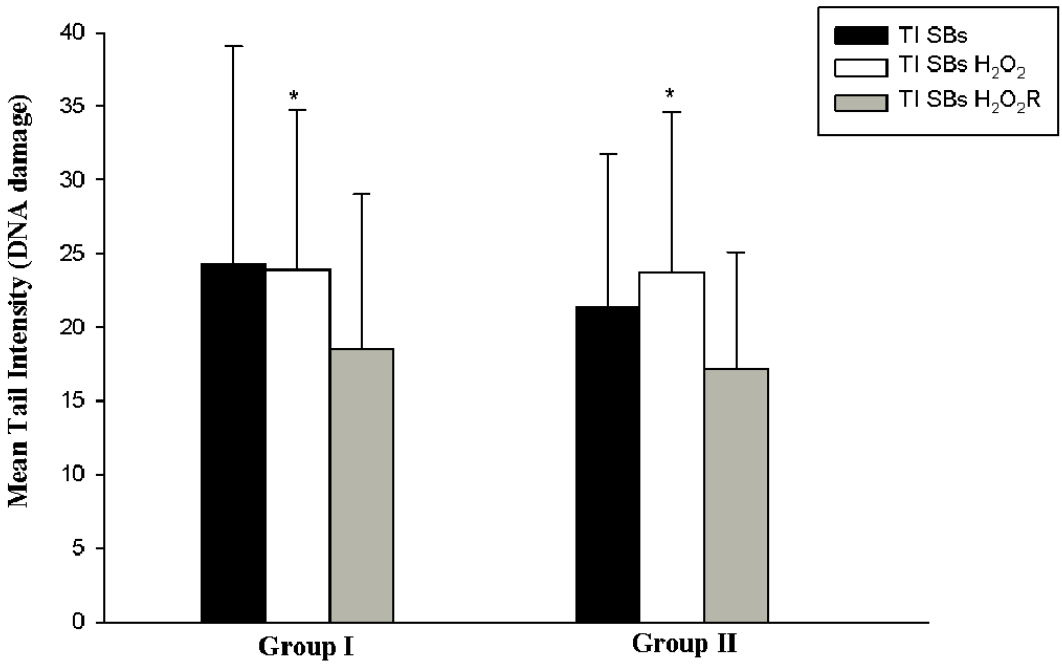

Figure 2

Influence of *micronutrients* on DNA damage (Tail Intensity).

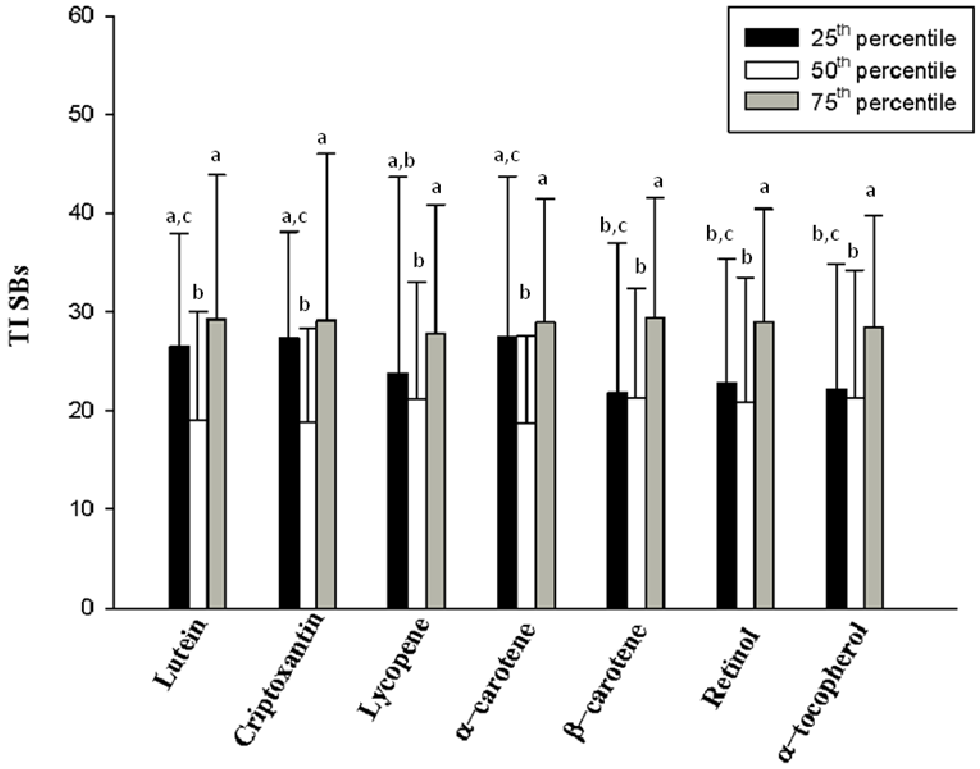

Figure 3

Influence of *micronutrients* on oxidized purines (Tail Intensity).

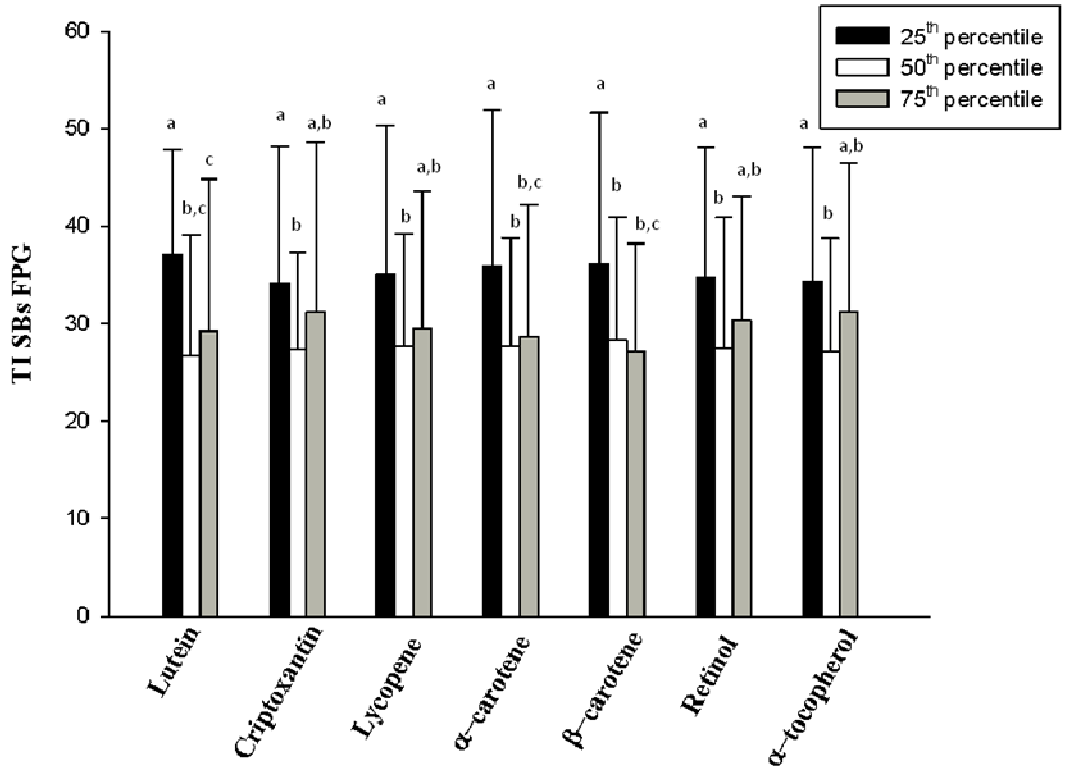

Figure 4

Influence of *micronutrients* on oxidized pyrimidines (Tail Intensity).

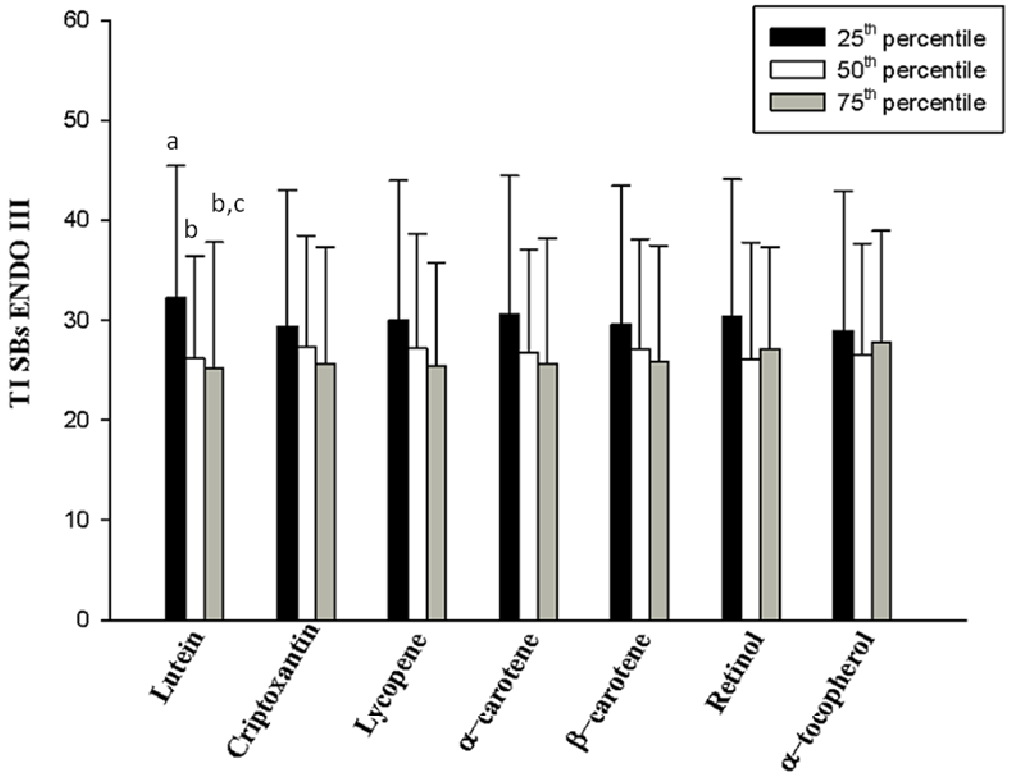

Figure 5

Influence of *micronutrients* on the level of misincorporation uracil (Tail Intensity).

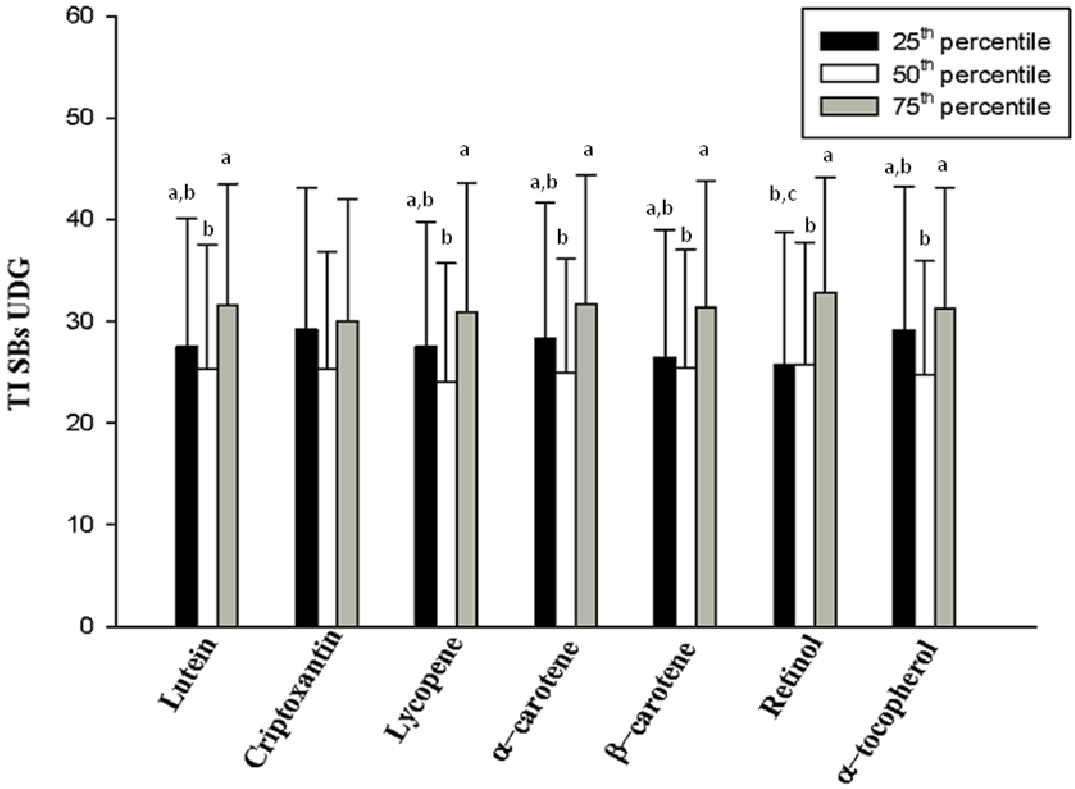

Figure 6

Influence of *micronutrients* on DNA damage induced by H_2O_2 (Tail Intensity).

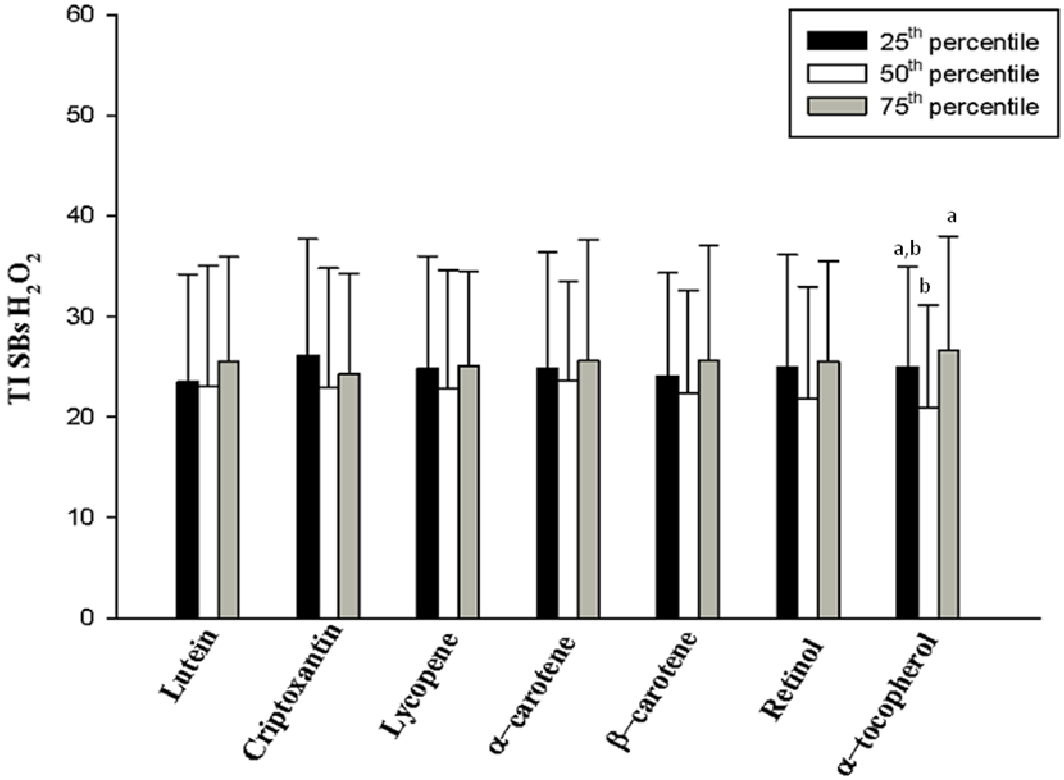

Figure 7

Influence of *micronutrients* on the level of DNA repair capability (Tail Intensity).

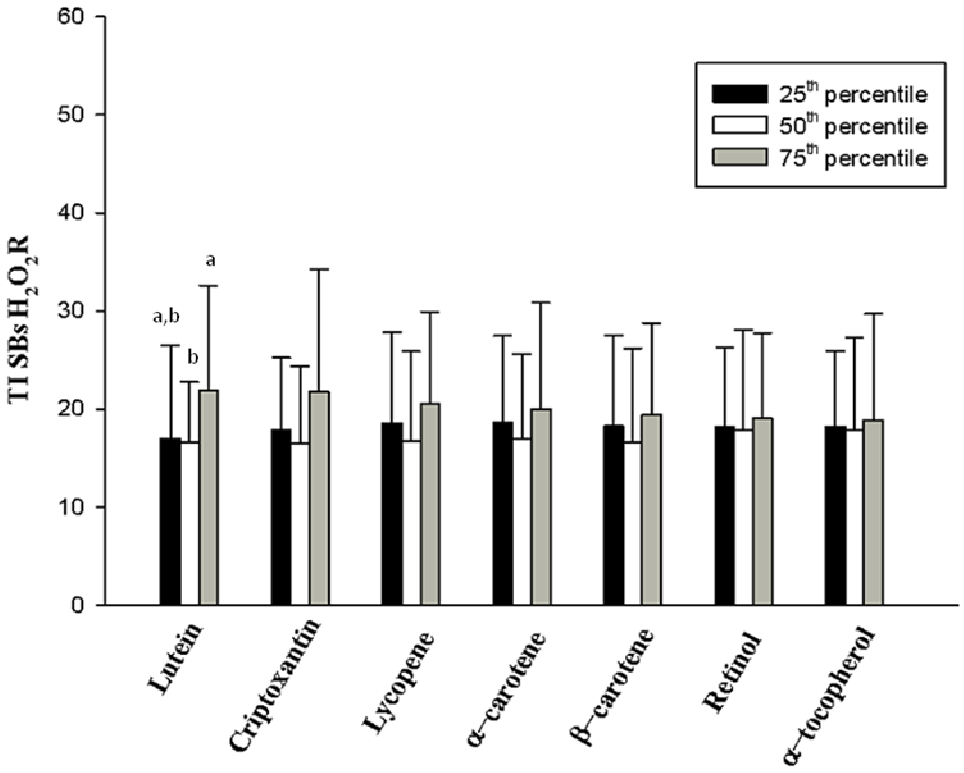

Figure 8

Manuscrito II

Trabalho elaborado segundo as normas da revista “The Journal of Nutrition”.

Genetic susceptibility to DNA damage in two populations under different dietary patterns

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(*Running Title*): Diet, gene polymorphism, and DNA damage and repair

Key Words: Comet assay, Diet, DNA repair, oxidative DNA damage, uracil misincorporation and polymorphisms.

Abstract

Diet is important factor in the etiology of various chronic degenerative diseases Possibly, due to the low ingestion of micronutrients and the high ingestion of chemical addictive presented in the modern diet, which associated to the genetic factors, could lead to genomic instability. The present study evaluated the influence of polymorphisms of the genes *XRCC1 (in codons 194 and 399,* rs1799782 and rs25487, respectively), *MTHFR (C677T and A1298C,* rs1801133 and rs1801131, respectively)*, CYP1A1* (rs1048943*) and GSTP1* (rs1695) on the level of oxidative DNA damage and uracil incorporation, efficiency of DNA repair in lymphocytes of two groups of individuals with different diets. GI: 87 individuals with diet rich in organic products, integral grains, fruit and vegetables and poor in industrialized products; GII: 97 individuals with diet rich in industrialized products and poor in fruit and vegetables. Oxidative DNA damage, uracil incorporation and the efficiency of DNA repair were assessed by the Comet assay and polymorphisms by PCR real time. With regard to individuals of GI: a) carriers of *GSTP1 AG* genotype presented higher levels of DNA damage induced by H_2O_2; b) carriers of *XRCC1 194 CC* genotype presented higher levels of single and double strand breaks and labile sites, c) carriers of *XRCC1 194 CC* and *XRCC1 194 TC* genotypes presented lower DNA repair capability; and d) carriers of *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of oxidized pyrimidines. With respect to individuals of the GII: a) *XRCC1 194 TT* and *XRCC1 194 TC* genotypes presented higher levels of oxidized pyrimidines; b) carriers of the *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of misincorporated uracil in DNA. In conclusion, polymorphisms of genes of metabolism and DNA repair could modulate levels of DNA damage and efficiency of DNA repair.

Introduction

Diet has long been studied as a potentially important factor in the etiology of various chronic degenerative diseases [1]. Malnutrition (excess or deficiency of micronutrients) itself may affect gene expression and genome stability, the latter leading to mutations in the gene sequence or chromosomal level that can cause abnormal gene dosage and expression of the gene leading to phenotypes adverse (diseases) during the various stages of life [2]. This can happen because these foods are sources of antioxidants (such as vitamins and minerals), which are substrate and/or cofactors in the several biologic pathways and, if not ingested in a proper concentration, they may impair enzyme activity necessary for regulate genomic stability [3; 4] and thus can induced DNA damage that is similar extents as those DNA damage that are induced by significant exposure to known carcinogens, such as ionizing radiation [4].

However, it is well known that certain chronic degenerative diseases, such as cancer, may be related to an interaction between environment (diet) and genetic factors, since the optimal concentration of micronutrients for the prevention of genomic damage is also dependent genetic polymorphisms that alter the function of genes involved directly or indirectly in the absorption and metabolism of micronutrients required for repair and replication of DNA [3]. Among these genetic polymorphisms, most significantly studied include genetic differences in biometabolism reaction dynamics to xenobiotic agents, in metabolism of one-carbon [5] and in the capacity to repair and replication of DNA [6].

Regarding the polymorphisms of enzymes involved in activating and detoxifying xenobiotic agents, there are basically two types of enzymes involved in chemical compound metabolism: Phase I codified by the cytochrome P450 (CYPs) gene super family, such as *CYP1A1*, which through oxidation reactions can transform pro-

carcinogens into carcinogenic substances capable of interacting with DNA [7; 8], inducing different types of damage, and Phase II, such as glutathione s-transferases (GSTs) which act on the metabolism of certain toxic substances, making them more hydrophilic and therefore capable of being more easily excreted [9]. GSTs in humans are polymorphic and an important gene is *GSTP1* which plays a fundamental role in protecting the organism against various types of cancer, since it is codified as an enzyme which acts in detoxifying polycyclic aromatic hydrocarbonates (PHA) originating from partially burnt organic material [10] and several chemical compounds found in processed foods. This enzyme has a single nucleotide polymorphism (SNP), which causes an isoleucine to valine substitution at position 313, making the enzyme less active favouring accumulation of DNA damage [10]. This polymorphism is associated with an increased risk of developing bladder, testicular, prostate [11], pharyngeal, laryngeal, and lung cancer [12].

Another genetic variability has been associated with genomic instability are the polymorphism of the gene *MTHFR*. This gene encodes an enzyme, which is involved in the carbon metabolism, the methylenetetrahydrofolate reductase (MTHFR), which catalyses the conversion of 5,10-methylenetetrahydrofolate into 5-methylenetetrahydrofolate, the primary circulating form of folate [13]. Regarding to this gene, the most extensively studied SNPs studied are at base pair 1298 (when adenine is replaced by cytosine, variant changes a glutamate to alanine) that is associated with reduced enzyme activity in homozygotes [14] and at base pair 677 (when cytosine is replaced by thymine, which yields a replacement of alanine with valine) whose individuals who are heterozygous (*CT*s) and homozygous (*TT*s) for this *MTHFR* polymorphism have as *in vitro* enzyme activity that is 30 and 65% of *CC*, respectively [15; 16]. The *A1298C* was first associated with ovarian carcinoma [17], and studies

have demonstrated that *A1298C* polymorphism of *MTHFR* is associated with a reduction risk of acute lymphoblastic leukemia in adults and children carrying the mutant allele in heterozygosity (*1298AC*) or homozygosity (*1298CC*) [18; 19]. *TT* genotype for the *C677T* variant is associated with an elevated risk for cardiovascular disease, which can be linked with homocysteine levels [20], and are reported to have higher risk for endometrial cancer [21]. The metabolism of one carbon needs folic acid that provides methyl groups for the methionine synthase-mediated remethylation of homocysteine to methionine [13], which is the immediate precursor of S-adenosylmethionine, the principal methyl donor for methylation reactions, including DNA methylation [22; 23]. Moreover, the homocysteine accumulate due to deficiency of folic acid could lead to reactive oxygen species (ROS) formation, with consequent oxidative stress that could lead to several diseases as cardiovascular diseases and cancer [24]. In addition, in conditions of folic-acid deficiency decreasing the availability of N5,N10- ethylenetetrahydrofolate, which is needed for the *de novo* synthesis of thymine, leads to the misincorporation of uracil into DNA that, in excess, not only generates point mutations, but can also cause single- and double-strand breaks, that could lead to the genomic instability [13;25].

Several investigations have demonstrated different individual capability to repair DNA. In fact, allelic variants of DNA repair gene, in conjunction with place of residence, have been associated with the increased risk for cancer [26]. The X-ray repair cross-complementing group 1 (*XRCC1*) gene is involved in the base excision repair (BER) [27]. The XRCC1 protein acts as a scaffolding protein that directly interacts with OGG1, poly (ADP-ribose) polymerase (PARP), DNA ligase III, and DNA polymerase b, facilitating the repair of single base modifications, nonbulky adducts, oxidative DNA damage, alkylation adducts, and damage induced by ionizing radiation [28]. More than

60 validated SNPs in the *XRCC1* gene has been listed in the Ensembl database [29]. However the most extensively studied SNPs are *Arg194Trp* (*C26304T*) in exon 6 and *Arg399Gln* (*G28152T*) in exon 10 [29]. The functional significance of these *XRCC1* variants has not been elucidated, but some of the polymorphisms are associated with a reduced repair capacity, consequent DNA damage accumulate and increased susceptibility to adverse health conditions, including cancer and coronary atherosclerosis [29; 30].

Therefore, based on these studies and evidences, in the present study we evaluated the influence of *XRCC1* allelic variations (in codons 194 and 399), *MTHFR* allelic variations (*C677T* and *A1298C)*, *CYP1A1* and *GSTP1* on the level of oxidative DNA damage, uracil misincorporation and DNA repair capability in peripheral blood lymphocytes of two groups of individuals with antagonist dietary patterns.

2. Materials and Methods

The present study was developed in Botucatu Medical School-UNESP, Internal Medicine Department and Pathology Departament, in Botucatu, State of São Paulo, Brazil. This study was approved by the local Ethical Committee. Informed consent to participate was obtained from all the volunteers.

2.1. Volunteers

A total of 202 healthy adult volunteers were interviewed. From these volunteers, a group of 18 individuals were excluded from the study due to technical problems or because did not fit in the parameters established. Therefore, 184 healthy adult volunteers (ranging from 18 and 66 years), 72 men and 112 women, were evaluated in this study, which is a continuation of a previous study [31]. All of the volunteer were

nonsmokers, were not abusing alcohol, and were not using supplementation, prescription or recreational drugs.

All participants donated a blood sample and an informed consent to participate was obtained from all the volunteers. The volunteers were divided in two groups for analysis of genome damage in lymphocytes: 1) 87 naturalistic individuals with a uncommon life style characterized by absence of urban stress, bucolic life and great consume of organic fruits, vegetables and juice and poor consume of industrialized foods (Group I); 2) 97 individuals with great ingestion of industrialized foods and poor ingestion of fruits and vegetables (Group II).

Only were selected individuals that have adopted the respective diet pattern for at least 10 years.

2.2. Dietary Assessment

For evaluation of the dietary pattern a Food-frequency Questionnaire adapted from Cardoso and Stocco [32] was applied, and was used for the classification of the groups. For calculating the estimated level of micronutrients ingested, a dietary record of three days was applied, and has provided data, which were inserted in the program Avanutri (AvaNutri Informática Ltda, RJ, Brazil). This procedure enabled us to establish an estimate actual quantity of protective foods consumed daily by the population studied. To be classified in Group I, the individuals had to consume the percentile 75 of organic fruits and vegetables and below the percentile 25 of each industrialized products. The individuals of group II were those who consumed less than percentile 25 of organic fruits and vegetables and above the percentile 75 of industrialized products.

2.3. *Laboratory analyses*

From each volunteer was collected, by trained personnel and using disposable material, 20 ml of peripheral blood. Of those, 2 ml were used to detect single and double strand breaks, labile sites (SBs), oxidative DNA damage, uracil misincorporation and DNA repair capability, which was measured by using the comet, 4 ml for extraction of DNA and RNA for determination of polymorphisms and gene expression profile, respectively (future studies), and 14 ml for micronutrients analysis, which was evaluated by high-performance liquid chromatography (HPLC) (future studies).

2.3.1. DNA extraction

Genomic DNA was isolated from peripheral blood leucocytes using a commecially available kit *Ilustra Genomic Prep Blood DNA Isolation* (Amersham Pharmacia Biotech Inc, USA), according to the manufacture's instructions. DNA extracted was stored at -20º C until genotyping.

2.3.1. Genotype analysis

The analyzes for the identification of single nucleotide polymorphisms (SNPs) of the *XRCC1* (in codons 194 and 399, rs1799782 and rs25487 , respectively), *MTHFR* (*C677T* and *A1298C,* rs1801133 and rs1801131 , respectively), *CYP1A1* (rs1048943) and *GSTP1*(rs1695) were performed using the TaqMan genotyping assay (Applied Biosystems), according to instructions the manufacturer. For the reaction of PCR (Polymerase Chain Reaction) were used aliquots of 10 µl, including 3 µl of DNA, 1,75 µl of ultrapure autoclaved H_2O, 5 µl of master mix (TaqMan® SNP Genotyping Master Mix – Applied Biosystems/USA) and 0.25 µl of primers (TaqMan ® SNP Genotyping Assays - Applied Biosystems / USA). DNA samples were amplified by polymerase chain

reaction in real time (ABI 7500 Applied Biosystems fast). The PCR reaction was performed in 40 cycles : 60° C for 1 minute, 95° C for 10 minutes, 95° C for 5 seconds, 60° C for 1 minute and 60° C for 1 minute. To assess the reproducibility of the genotyping, a random selection of 10% of the samples were genotyped again with 100% agreement.

2.3.2. Determination of Oxidative DNA Damage and level of uracil incorporate into DNA

Single and double strand breaks and labile sites (SBS), oxidative DNA damage, uracil misincorporation into DNA, and DNA repair were evaluated as described and accomplished by Prado et al in a previous study [31]. The alkaline Comet assay [33], modified with lesion-specific enzymes was used to detect SBs, oxidised purines and pyrimidines and uracil [34]. All procedures were conducted in the dark to minimize spurious sources of DNA damage. Briefly, 10 μl of the isolated lymphocytes suspension [35] (≅ 2 x 10^4 cells) were embedded into 0.5% low melting point agarose (Sigma) and spread on agarose-precoated microscope slides. Twelve slides were prepared for each sample. Slides were immersed overnight in freshly prepared cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris, 1% sodium salt N-Lauryl sarcosine, pH 10.0, with 1% Triton X-100 and 10% DMSO added fresh) at 4^0C. After lysing, the slides were washed three times in enzyme buffer (Trevigen, Gaithersburg, MD) and then three slides for each treatment were incubated at 37°C for 45 min with 100 μL of endonuclaese III (Endo III - 1:1000; New England Biolabs Inc), 100 μL of formamidopyrimidine-DNA glycosylase (FPG - 1:1000; New England Biolabs Inc), 100 μL of uracil-DNA glycosylase (UDG - 1:1000; New England Biolabs Inc) or with enzyme buffer only. Endo III recognizes oxidised pyrimidines (SBs EndoIII), while

FPG identifies oxidised purines (SBs FPG), especially 8-oxo-guanine and UDG recognizes uracil (SBs UDG). Enzyme buffer only is used to identified SBs. Subsequently the cells were exposed to alkali buffer (1mM EDTA and 300 mM NaOH, pH ≅ 13.4), at 4°C, for 40 min to allow DNA unwinding and expression of alkali-labile sites. Electrophoresis was conducted in the same solution at 4°C, for 30 min, at 25 V (1v/cm) and 300 mA. After electrophoresis, the slides were neutralized (0.4 M Tris, Ph 7.5), stained with 50 µL Syber Green (1:10000; Invitrogen), and analyzed in a fluorescence microscope at 400 x magnification, using an image analysis system (Comet Assay II – Perceptive Instruments, Suffolk, UK).

For each individual, one hundred randomly selected cells (50 from each of two replicate slides) for each treatment (FPG, Endo III, UDG or enzyme buffer only) were evaluated and the mean tail intensity (%DNA Tail) was determined, totaling 400 cells analyzed from each individual. Tail Intensity, according to Comet Assay II – Perceptive instruments, is defined as "the sum of all intensity values in the tail region less those which are part of the mirrored head region. The value is also expressed as a percentage of the total Comet or cell intensity".

To analyze the influence of dietary patterns and all of polymorphisms on the DNA repair capability, 200 µl of the freshly isolated lymphocytes were treated with 100 µl of H_2O_2 (100 µM), for 30 minutes, in ice, and analysis of the level of damage was evaluated. Then, after two wash in PBS to remove of H_2O_2, these aliquots were incubated at 37 °C for 30 minutes, and DNA damage was checked again.

2.4. Statistical Analysis

The data obtained in the Comet Assay had asymmetric distribution, then a nonparametric analysis was applied. For evaluate the influence of all polymorphisms on the various types of DNA damage, the Kruskal-Wallis Test was applied. The index of significance adopted was 5%.

3. Results

DNA damage, including single and double strand breaks, labile sites, oxidised purines and pyrimidines and misincorporated uracil on DNA, were detected by the alkaline Comet assay modified with lesion-specific enzymes, FPG, Endo III and UDG, respectively. The enzyme-modified assay measures oxidative DNA damage as a combination of SBs and oxidised bases: formamidopyrimidines and the oxidised purine 8-oxo-guanine when considering FPG (SBs FPG), or oxidised pyrimidine when considering endonuclease III (SBs Endo III). The enzyme-modified assay also measures misincorporated uracil on DNA when considering use of uracil-DNA glycosylase (SBs UDG).

We observed that 65 individuals in group I and 58 individuals in group II carry the *CYP1A1 AA* genotype; 18 individuals in group I and 20 individuals in group II carry the *CYP1A1 AG* genotype; and 4 individuals in group I and 19 individuals in group II carry the *CYP1A1 GG* genotype. Based on this, we calculate the allele and genotype frequencies of *CYP1A1* gene that are presented in Table 1. Only the individuals in group II are not in Hardy-Weinberg equilibrium for this gene. *CYP1A1 AA* genotype was more frequent in individuals of the group I than in group II, but the *CYP1A1 GG* genotype was more frequent in individuals of the group II than in group I. As regards the allelic frequency, the A allele was more frequent in group I compared to group II; conversely,

in group II the G allele was more frequent. In addition, we evaluated the influence of *CYP1A1* polymorphisms on levels of SBs, oxidative DNA damage, misincorporation uracil on DNA, DNA damage induce by H_2O_2 (100 μM) and on the DNA repair capability in each group that was showed in Figure 1. None of the genotypes of *CYP1A1* genes influenced the baseline levels of single and double strand breaks, labile sites, oxidised purines and pyrimidines and misincorporated uracil on DNA, DNA damage induce by H_2O_2 and the DNA repair capability, in both groups.

We also observed that 36 individuals in group I and 53 individuals in group II carry the *GSTP1 AA* genotype; 43 individuals in group I and 36 individuals in group II carry the *GSTP1 AG* genotype; and 8 individuals in each group carry the *GSTP1 GG* genotype. Table 2 shows the genotypic and allele frequencies of the *GSTP1* gene. Both populations studied are in Hardy-Weinberg equilibrium for this gene. Genotypic frequency evaluation demonstrated that the *GSTP1 AA* genotype was more frequent in individuals of the group II than in group I, while the *GSTP1* AG genotype was more frequent in individuals of the group I than in individuals of group II. The frequencies of alleles A and G were statistically different ($p < 0.01$) within each group (allele A was the most common), but not between groups. However, the individuals of group I carrying *GSTP1 AG* genotype presented higher levels of DNA damage induced by H_2O_2 than individuals carrying *GSTP1 AA* and *GSTP1 GG* genotypes (Figure 2).

Regarding to the polymorphism of the gene *MTHFR*, we observed in the group I that 53 individuals carry the *MTHFR 1298 AA* and 34 individuals carry *MTHFR 677 CC* genotype; 34 individuals carry the *MTHFR 1298 AC* and 47 individuals carry *MTHFR 677 CT* genotype; and 6 individuals carry *MTHFR 677 TT* genotype. On the other hands, we observed in the group II that 63 individuals carry the *MTHFR 1298 AA* and 35 individuals carry *MTHFR 677 CC* genotype; 26 individuals carry the *MTHFR 1298*

AC and 48 individuals carry *MTHFR 677 CT* genotype; 8 individuals carry the *MTHFR 1298 CC* and 14 individuals carry *MTHFR 677 TT* genotype. Genotypic and allele frequencies of the *MTHFR* genes are in the Table 3 and 4. Both populations studied are in Hardy-Weinberg equilibrium for the *MTHFR C677T* gene, but we do not observe the Hardy-Weinberg equilibrium in the population of the group II for the *MTHFR A1298C* , although we found when we analyze the equilibrium in the population as a whole. *MTHFR 1298 AC* genotype was more frequent in individuals of the group I than in individuals of the group I (Table 3). None difference between groups, but the frequency of allele A was higher than frequency of allele C ($p < 0.01$) within each group (Table 3). Moreover, *MTHFR 677 CT* genotypes were more frequent in both groups and the frequencies of alleles C and T were statistically different ($p < 0.01$) within each group (allele C was the most common), but not between groups (Table 4). Furthermore, none of the genotypes of *MTHFR A1298C* and *MTHFR C677T* genes influenced the baseline levels of single and double strand breaks, labile sites, oxidised purines and pyrimidines and misincorporated uracil on DNA, DNA damage induce by H_2O_2 and the DNA repair capability, in both groups (Figure 3 and 4, respectively).

Table 5 shows the genotypic and allele frequencies of the *XRCC1 194 Trp* polymorphism. Both populations studied are in Hardy-Weinberg equilibrium. We found 48 individuals in group I and 64 individuals in group II carry the *XRCC1 194 TT* genotype; 32 individuals in group I and 27 individuals in group II carry the *XRCC1 194 TC* genotype; and 7 individuals in group I and 6 individuals in group II carry the *XRCC1 194 CC* genotype. *XRCC1 194 TT* genotype was more frequent in individuals of the group II than in group I, at the same time as the *XRCC1 194 TC* genotype was more frequent in individuals of the group I than in individuals of group II. The frequency of allele C was lower than frequencies of allele T ($p < 0.01$) within each group, but not

between groups. In addition, individuals in the group I carrying the *XRCC1 194 CC* genotype presented higher levels of single and double strand breaks and labile sites than individuals carrying the *XRCC1 194 TT* and *XRCC1 194 TC* genotype (Figure 5). Individuals in the group I carrying the *XRCC1 194 TC* genotype presented higher levels of single and double strand breaks and labile sites than individuals carrying the *XRCC1 194 TT* genotype and the individuals carrying the *XRCC1 194 CC* and *XRCC1 194 TC* genotypes presented lower DNA repair capability than individuals carrying the *XRCC1 194 TT* genotype. While in the group II, individuals carrying the *XRCC1 194 TT* genotype presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 194 TC* and *XRCC1 194 CC* genotypes. Moreover, individuals carrying the *XRCC1 194 TC* genotype presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 194 CC* genotypes (Figure 5).

We also evaluated the genotypic and allele frequencies of the *XRCC1 399Gln* gene (Table 6). Both populations studied are in Hardy-Weinberg equilibrium for this gene. We found 40 individuals in group I and 33 individuals in group II carry the *XRCC1 399 GG* genotype; 38 individuals in group I and 42 individuals in group II carry the *XRCC1 399 GA* genotype; and 8 individuals in group I and 22 individuals in group II carry the *XRCC1 399 AA* genotype. Based on this, we note that the *XRCC1 399 GG* genotype was more frequent in individuals of the group I than in group II. Furthermore, the *XRCC1 399 AA* genotype was more frequent in individuals of the group II than in individuals of group I. Regarding to the allelic frequency, the G allele was more frequent in Group I compared to Group II; inversely, in Group II the A allele was more frequent. Therefore, we observed in this figure that the individuals of group I carrying the *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 399 GA* genotype (Figure 6). In

addition, individuals of group I carrying the *XRCC1 399 AA* genotypes presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 399 GG* genotype. While in Group II, the individuals carrying the *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of misincorporated uracil in DNA than individuals carrying the *XRCC1 399 GA* genotype (Figure 6).

4. Discussion

Diet certainly plays an important role in chronic degenerative diseases as cancer, diabetes mellitus II, Alhzeimer, Parkinson, infertility, immune dysfunction, and cardiovascular diseases [1; 36; 37; 38;]. Several in vitro studies has been demonstrated that some micronutrients present in the diet (carotenoids and vitamins) have an antioxidant potential, that show a protective effect, since they act as a first line of cellular defense against DNA oxidation [39]. On the other hands, we know that food mutagens could cause different types of DNA damage leading to nucleotide alterations and gross chromosomal aberrations, but the effects of food mutagens in carcinogenesis can be modified by heritable traits, low-penetrant genes that affect mutagen exposure of DNA through metabolic activation and detoxification or cellular responses to DNA damage through DNA repair mechanisms or cell death [40]. However, the participation of each one of these factors in the development of chronic degenerative diseases has not yet been fully clarified. In a previous study performed in our laboratory [31], we have shown that individuals with a diet rich in manufactured products and poor in vegetables have higher levels of purines and pyrimidines oxidized and higher levels of DNA damage induced by H_2O_2 than individuals with a diet rich in natural products, such as vegetables and grain. Nevertheless, the data presented a high heterogeneity suggesting

the evolvement of other factors. Therefore, in this study we investigate if some gene variants, involved in metabolism xenobiotic (CYP1A1 and GSTP1), in one-carbon pathway metabolism (MTHFR A1298C and MTHFR C677T) and in the capacity to repair DNA (XRCC1 194 Trp and 399Gln), are related to increasing the level of DNA damage, which could be a precursor of chronic degenerative diseases [39].

In the last years, several studies have explored the influence of SNPs on DNA metbolism xenobiotic genes and the interaction of these genotypes on genotoxic exposure biomarkers [41]. The determination of SNP is important aspect that may increase sensitivity and specificity of assays and identify effects and susceptible individuals and subgroups [40; 41]. Although in several studies *CYP1A1* polymorphisms have been associated with higher levels of genotoxic damage and risk development of cancer [40], we did not found influence of none genotypes of *CYP1A1* gene on the baseline levels of single and double strand breaks, labile sites, oxidised purines and pyrimidines and misincorporated uracil on DNA, DNA damage induce by H_2O_2 and the DNA repair capability, in both groups. Our results are in agreement with the results reported by Villarini et al. [42] that reported no effects of *CYP1A1* variants were observed for the primary and oxidative DNA damage analyzed in workers exposure to indoor pollutants during road tunnel construction. CYP1A1 variants also showed differential regulatory properties in the induction of expression and enzyme kinetics compared to the wildtype [43, 44], although some discrepancies remain among references. Schwarz et al. [45] reported a smaller enzyme activity of the *CYP1A1 Ile462Val* genotype (*CYP1A1 AG* and *GG*) when compared to *CYP1A1 AA* genoype, but these results disagreed with those of Kawajiri et al. [46] who found a higher activity in the variant form (*CYP1A1 GG*). *CYP1A1* is important for the activation of precarcinogens [47], whose the enzyme codified is involved in the bioactivation and

detoxification of a variety of xenobiotics present in food [48]. Perhaps we have not seen the influence of this polymorphism in the individuals of group I, due to the fact that these individuals have a diet poor in processed foods, and therefore, were not exposed to xenobiotics present in food. On the other hand, in group II, we also do not observe the influence of this polymorphism, possibly because the allele frequencies in this group was not in Hardy-Weinberg equilibrium, and some other factor may be acting, even they have shown a higher frequency of G allele, since the G allele is related to an increase in enzymatic activity [49]. In addition, the present study evaluated the influence of CYP1A1 polymorphism alone, but we believe that specific metabolism enzymes could be relationship with expression of other enzymes of the xenobiotic metabolism, acting in combination, which could explain the lack of influence of these genotypes in our results.

Despite a study developed by Tijhuis and colleagues [50] have demonstrated an increased risk for colorectal adenomas associated with higher cruciferous vegetable intake among those with the *GSTP1 GG* genotype, but not for those with one or two copies of the common A allele, in this study we showed that individuals of group I carrying *GSTP1 AG* genotype presented higher levels of DNA damage induced by H_2O_2 than individuals carrying *GSTP1 AA* and *GSTP1 GG* genotypes. Gene polymorphism in a π class GST, GSTP1, involves an amino acid substitution ($Ile^{105} \rightarrow Val^{105}$) resulting in decreased enzyme activity [51]. Some agents are metabolized by phase I and II enzymes, and the balance between these phases could determine the level of reactive oxygen species (ROS) that could react with DNA induce DNA damage. Although the individuals in the group I do not have a high intake of processed foods, *GSTP1* gene codified an enzyme which acts in detoxifying PHA originating from the incomplete combustion of organic matter during cooking of meat, fish and vegetables [10; 52], that

are present in the diet of the group I (data not shown), that after Phase I metabolism could generate ROS that induce DNA damage. However, we observed an increase in the level of damage to DNA damage only with regard the damage induced by hydrogen peroxide (H_2O_2) in these individuals. Perhaps, ROS generate by H_2O_2 plus ROS generate by the metabolism of PHA (excess of ROS in this individuals) could be the reason of our results. In addition, we observed that *GSTP1* AG genotype was more frequent in individuals of the group I than in individuals of group II, with a frequency of 0.49. Perhaps this more frequency in group I can explain the fact that we only have found damage increase in the group I. Our results are in agreement with the results reported by Magno et al., [53] that reported the most frequent genotype for *GSTP1* (rs1695) was the heterozygous form in a Brazilian Population, with 46.6% . However, we did not find a higher level of DNA damage induced by H_2O_2 in individuals of group I carrying *GSTP1 GG* genotypes, possibly due to the small number samples, just 8 individuals in each group.

Another marked evaluated in this study showed that *MTHFR 677 CT* genotypes were more frequent in both groups, around 50%. In Europe, 8–20% of the Caucasian population is homozygous for the *677T* allele and almost 40% is heterozygous [54]. Moreover, we observed that the C allele frequency of *MTHFR 1298* in this study was 0.21 to group I and 0.22 to group II. Similar results were reported by Lordelo et al., [55] where the C allele frequency of *MTHFR 1298* ranges from 0.17-0.19 among Asians to 0.27-0.36 in Western Europe and in a Brazilian population of healthy individuals was around 0.30. Furthermore, none of the genotypes of *MTHFR A1298C* and *MTHFR C677T* genes also influenced the baseline levels of single and double strand breaks, labile sites, oxidised purines and pyrimidines and misincorporated uracil on DNA, DNA damage induce by H_2O_2 and the DNA repair capability, in both groups. Reports showed

that *1298CC* genotype has been found to have 60% of the 1298AA wild-type enzyme activity *in vitro* [56], even as individuals heterozygous (*CT*s) and homozygous (*TT*s) for *MTHFR C677T* polymorphism have as *in vitro* enzyme activity that is 30 and 65% of normal, respectively [57; 58]. Although is reported that *1298CC* variant affects enzyme activity to a lesser degree than *677TT* variant [14], the reduced *MTHFR* activity due to these polymorphisms may increase 5,10-methylenetetrahydrofolate, that is essential to conversion of dUMP to dTMP for DNA synthesis and repair [59]. More 5,10-methylenetetrahydrofolate levels could increase the availability of thymine, reduce the incorporation of the former base into DNA and consequently DNA damage, and promote DNA stability [58; 60]. However, such protection of *MTHFR A1298C* and *MTHFR C677T* polymorphisms occurred with adequate folate intake. Although dietary intakes of both groups are completely different, even the group II consumed folate, in lesser amount, but should have been enough to promote DNA stability, which could explain our results and the lack of influences of these polymorphisms on biomarkers of DNA damage analyzed by us, in both groups. In addition, it is noticed that individuals of our study were all healthy subjects, nonsmokers, were not users of drugs, not users of medication for at least 30 days, were not abusing alcohol, and the majority was young individuals, what can contribute to these results. Others studies found similar results, as Narayanan et al [61], that find that lymphocyte DNA stability biomarkers were similar for all *MTHFR C677T* or *A1298C* variants. In another human study, all *MTHFR C677T* variants did not influence on DNA strand breakage, micronuclei frequency formation and chromatid exchange [62]. Moreover, a study *in vitro* found that *MTHFR C677T* or *A1298C* genotypes did not influence levels of chromosome damage measured by the cytokinesis block micronucleus assay [63].

We also note that *XRCC1 194 TT* genotype was more frequent in both groups, but higher in individuals of the group II than in group I. The distribution of *XRCC1 194Trp* polymorphisms found by us was similar to that found in São Paulo and Rio de Janeiro, the wild-type genotypes (*XRCC1 194 TT)* being the most frequent [64]. In addition, several studies have reported that the frequency of the 399Gln variant allele (A allele) was of 0.30 in Brazilians of European descent [64], or 0.32-0.37 for American Caucasians and 0.32-0.48 for European Caucasians [65; 66; 67; 68; 69; 70]. We found an allelic frequency of A allele similar to these studies, 0.31 to group I and 0.44 to group II.

Regarding to the influence of polymorphisms of the gene *XRCC1* (*194Trp* and *399Gln*) on DNA damage level, we observed that individuals in the group I carrying the *XRCC1 194 CC* genotype presented higher levels of single and double strand breaks and labile sites than individuals carrying the *XRCC1 194 TT* and *XRCC1 194 TC* genotype, and the individuals carrying the *XRCC1 194 CC* and *XRCC1 194 TC* genotypes presented lower DNA repair capability than individuals carrying the *XRCC1 194 TT* genotype. Also in Group I, individuals carrying the *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 399 GA* genotype. In addition, individuals of group I carrying the *XRCC1 399 AA* genotypes presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 399 GG* genotype. The *194Trp* codon of *XRCC1* is located in a highly conserved hydrophobic linker region between its DNA polymerase β domain and poly (ADP-ribose) polymerase-interacting domains, so the change from arginine to tryptophan (change from T allele to C allele) could alter the interaction of *XRCC1* with either or both of these DNA repair proteins within the base excision repair complex [71], leading to a lower efficiency enzymatic, which could explain our results.

Similarly, *XRCC1399Gln* polymorphism occurs in the interaction site with PARP (poly(ADP-ribose) polymerase). This exchange of base (G→A) may lead to a modification in repair activity, and XRCC1 Gln399 allele (A allele) has been reported as a risk factor for different types of cancer [72]. Our findings are in agreement with ref on the *XRCC1 194Trp* polymorphism, that shows significantly higher frequency of chromatid exchanges, estimated by assay sensitivity mutagenic than those with the wild-type 194Arg allele (T allele) [73]. Furthermore, the presence of the *Gln399Gln* genotype (*XRCC1 399 AA*) has been associated with persistence of DNA damage, elevated formation of sister chromatid exchange (SCE) [74] and baseline DNA damage in healthy individuals [75].

On the other hand, Wang et al [76] reported that patients with the wild-type Arg194Arg genotype (194 TT genotype) showed higher levels of DNA damage after exposure of lymphocytes to bleomycin, benzo[a]pyrene- diolepoxide than patients with C allele, suggesting its protective role against genotoxic treatment, as well as Cornetta et al. [72] which reported higher DNA damage level in individuals carrying of 399 GG genotype than in individuals carrying of *399 GA* or *399 AA* genotype. We also observed that, in group II (individuals more exposed to genotoxic agents from manufactured products), individuals carrying the *XRCC1 194 TT* genotype presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 194 TC* and *XRCC1 194 CC* genotypes. Moreover, individuals carrying the *XRCC1 194 TC* genotype presented higher levels of oxidized pyrimidines than individuals carrying the *XRCC1 194 CC* genotypes. We also observed that the individuals of group II carrying the *XRCC1 399 GG* and *XRCC1 399 AA* genotypes presented higher levels of misincorporated uracil in DNA than individuals carrying the *XRCC1 399 GA* genotype. However, we believe that without high intakes of antioxidants, oxidative DNA damage, mainly repaired by the

base excision repair pathway, may appear. Therefore, the increased levels of oxidized pyrimidines among the *194 TT* genotype carries may be due to increased redox activity and may have nothing to do with DNA repair. In addition, according Vineis [77] another possible explanation is that at high levels of genotoxic exposure, the DNA repair capacity of each individual may be saturated even if they have a higher efficiency enzymatic (*XRCC1 194 TT* and XRCC1 399 GG genotypes). Moreover, according with Cornetta et al. [72] the authors suggest that due to lower DNA repair capability induced by *XRCC1 399Gln* allele, damaged cells could accumulate a high DNA damage level and likely to be driven apoptosis, but how apoptosis/necrosis cells were excluded from our analysis, this can help to explain the higher levels of oxidative DNA damage, misincorportion uracil found in individuals carrying *XRCC1 399AA* genotype.

In conclusion, polymorphisms of genes of metabolism of xenobiotic (*GSTP1*) and DNA repair (*XRCC1 194Trp and 399Gln*) could modulate levels of DNA damage and efficiency of DNA repair, especially in subjects with higher intake of micronutrients. However, we believe that further investigations are needed to clarify the mechanisms of these polymorphisms in combination with other genes and micronutrients status.

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Legends

Table 1 . Genotype and allele distribution for the *CYP1A1* gene (rs1048943) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column); # $p < 0.01$- significant difference between groups (Group I x Group II).

Table 2 . Genotype and allele distribution for the *GSTP1* gene (rs1695) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column); # $p < 0.01$- significant difference between groups (Group I x Group II).

Table 3 . Genotype and allele distribution for the *MTHFR A1298C* gene (rs1801131) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column); # $p < 0.01$- significant difference between groups (Group I x Group II).

Table 4 . Genotype and allele distribution for the *MTHFR C677T* gene (rs1801133) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column).

Table 5 . Genotype and allele distribution for the *XRCC1 194Trp* gene (rs1799782) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column); # $p < 0.01$- significant difference between groups (Group I x Group II).

Table 6 . Genotype and allele distribution for the *XRCC1 399Gln* gene (rs25487) in individuals with dietary patterns antagonistic. Different letters indicate statistical difference ($p < 0.01$) within the same group (column); # $p < 0.01$- significant difference between groups (Group I x Group II).

Figure 1. Influence of *CYP1A1* gene polymorphisms on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 μl of H_2O_2 (100 μM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5th and 95th percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Figure 2. Influence of *GSTP1* gene polymorphisms on DNA damage (Tail Intensity) on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 μl of H_2O_2 (100 μM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5th and 95th percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Figure 3. Influence of *MTHFR A1298C* polymorphisms on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs

H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 µl of H_2O_2 (100 µM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5^{th} and 95^{th} percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Figure 4. . Influence of *MTHFR C677T* polymorphisms on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 µl of H_2O_2 (100 µM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5^{th} and 95^{th} percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Figure 5. Influence of *194Trp* polymorphisms of DNA repair gene *XRCC*1 on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and

Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 µl of H_2O_2 (100 µM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5^{th} and 95^{th} percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Figure 6. Influence of *399Gln* polymorphisms of DNA repair gene *XRCC*1 on DNA damage (Tail Intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H_2O_2 and SBs H_2O_2R in peripheral blood lymphocytes from Group I (n=87) and Group II (n=97) individuals. SBs = single and double strand breaks and alkali labile sites; SBs FPG = SBs more FPG sensitive sites (purine oxidized); SBs Endo III = SBS more Endo III sensitive sites (pyrimidine oxidized), SBs UDG = SBs more misincorporated uracil sites, SBs H_2O_2 = level of DNA damage after treatment with 100 µl of H_2O_2 (100 µM), for 30 minutes, in ice and SBs H_2O_2R = level of DNA damage after incubation at 37 °C for 30 minutes. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5^{th} and 95^{th} percentiles at the whiskers. Tukey test was used to compare differences among genotypes in each group.

Table 1 . Genotype and allele distribution for the *CYP1A1* gene (rs1048943) in individuals with dietary patterns antagonistic.

	Group I	*Group II*
Genotypic frequency		
AA	$0.75^{a\#}$	0.60^{A}
AG	0.21^{b}	0.21^{B}
GG	0.05^{c}	$0.20^{B\#}$
Allele frequency		
A	$0.85^{a\#}$	0.7^{A}
G	0.15^{b}	$0.3^{B\#}$

Table 2 . Genotype and allele distribution for the *GSTP1* gene (rs1695) in individuals with dietary patterns antagonistic.

	Group I	*Group II*
Genotypic frequency		
AA	0.41^{a}	$0.55^{A\#}$
AG	$0.49^{a\#}$	0.37^{B}
GG	0.09^{b}	0.08^{C}
Allele frequency		
A	0.66^{a}	0.73^{A}
G	0.34^{b}	0.27^{B}

Table 3 . Genotype and allele distribution for the *MTHFR A1298C* gene (rs1801131) in individuals with dietary patterns antagonistic.

	Group I	***Group II***
Genotypic frequency		
AA	0.61^{a}	0.65^{A}
AC	$0.39^{b\#}$	0.27^{B}
CC		0.08^{C}
Allele frequency		
A	0.79^{a}	0.78^{A}
C	0.21^{b}	0.22^{B}

Table 4 . Genotype and allele distribution for the *MTHFR C677T* gene (rs1801133) in individuals with dietary patterns antagonistic.

	Group I	***Group II***
Genotypic frequency		
CC	0.39^{a}	0.36^{A}
CT	0.54^{b}	0.49^{B}
TT	0.07^{c}	0.14^{C}
Allele frequency		
C	0.66^{a}	0.61^{A}
T	0.34^{b}	0.39^{B}

Table 5 . Genotype and allele distribution for the *XRCC1 194Trp* gene (rs1799782) in individuals with dietary patterns antagonistic.

	Group I	***Group II***
Genotypic frequency		
TT	0.55^{a}	$0.66^{A\#}$
TC	$0.37^{a\#}$	0.28^{B}
CC	0.08^{b}	0.06^{C}
Allele frequency		
T	0.74^{a}	0.8^{A}
C	0.26^{b}	0.2^{B}

Table 6 . Genotype and allele distribution for the *XRCC1 399Gln* gene (rs25487) in individuals with dietary patterns antagonistic.

	Group I	***Group II***
Genotypic frequency		
GG	$0.46^{a\#}$	0.34^{A}
GA	0.44^{a}	0.43^{B}
AA	0.09^{b}	$0.23^{C\#}$
Allele frequency		
G	$0.68^{a\#}$	0.56^{A}
A	0.31^{b}	$0.44^{B\#}$

Influence of *CYP1A1* gene polymorphisms on DNA damage (Tail Intensity).

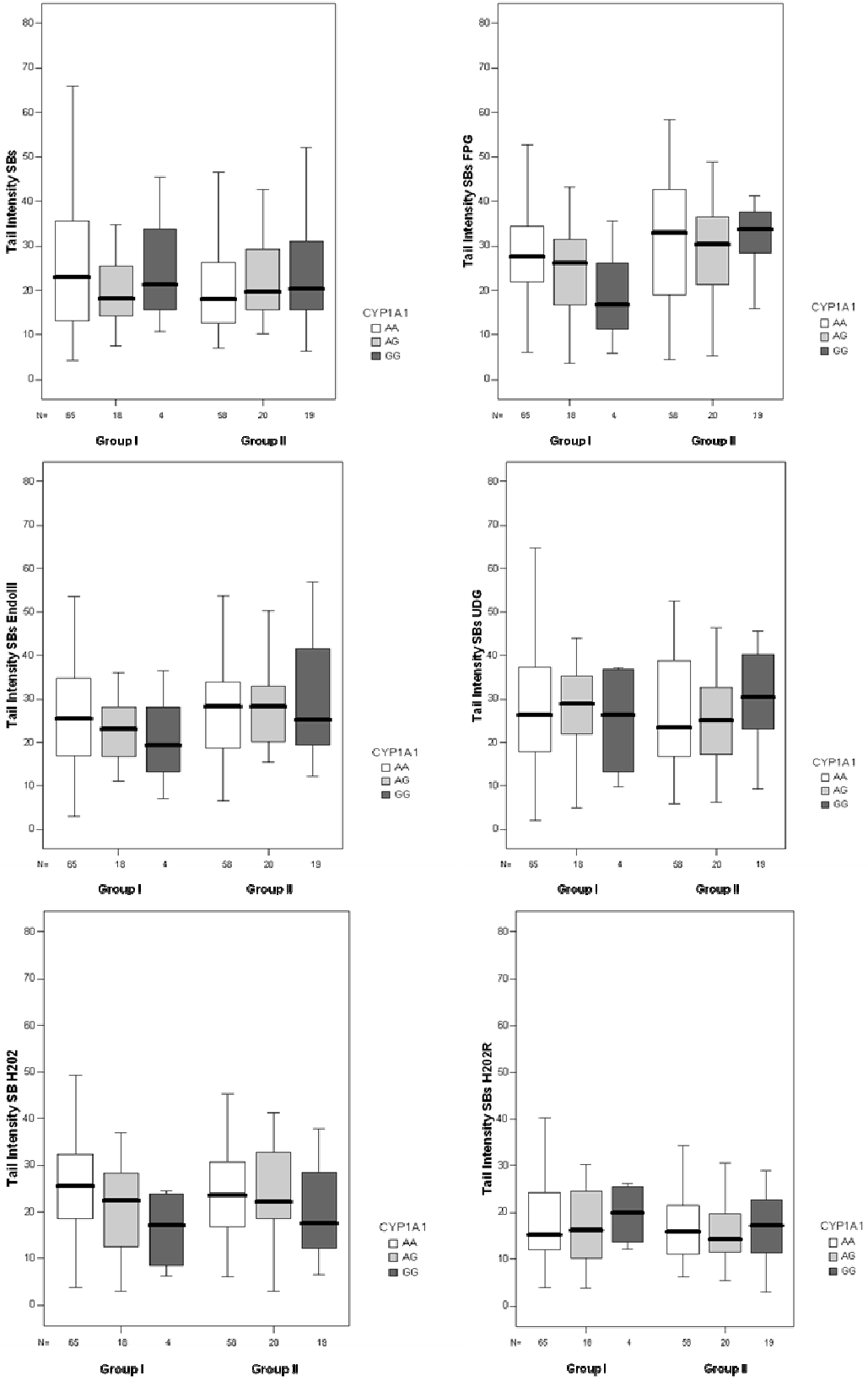

Figure 1

Influence of *GSTP1* gene polymorphisms on DNA damage (Tail Intensity).

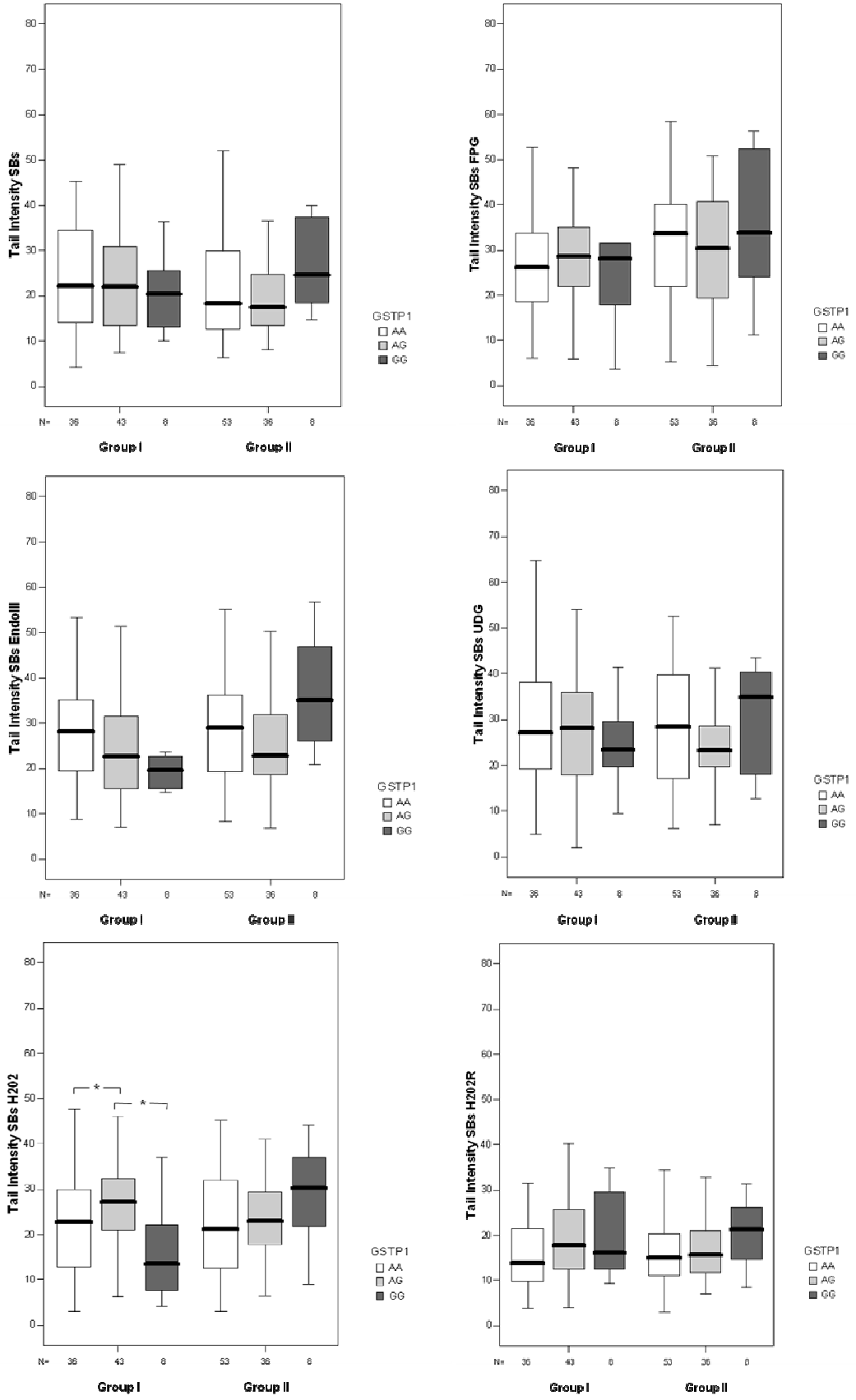

Figure 2

Influence of *MTHFR A1298C* gene polymorphisms on DNA damage (Tail Intensity).

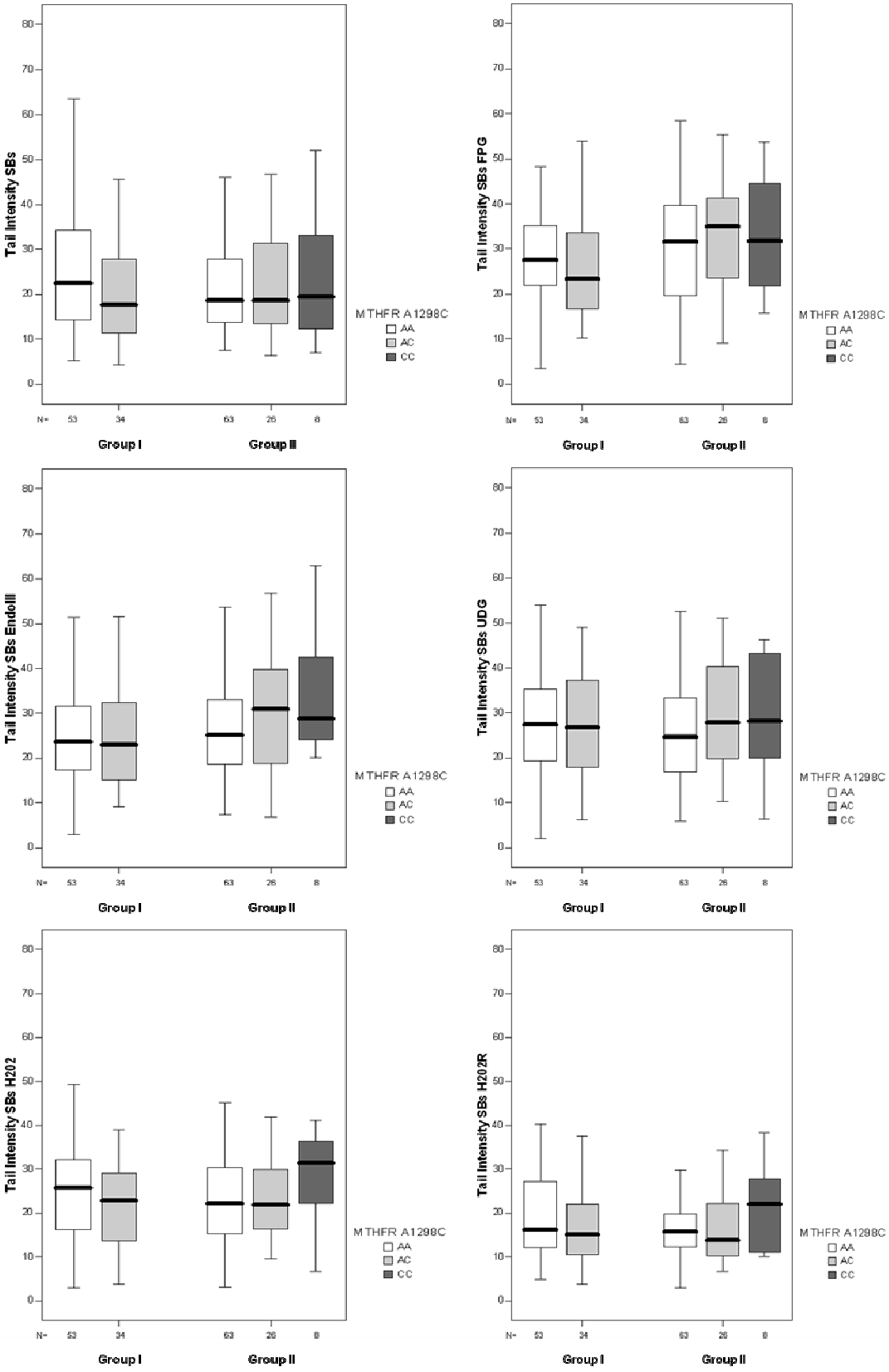

Figure 3

Influence of *MTHFR C677T* gene polymorphisms on DNA damage (Tail Intensity).

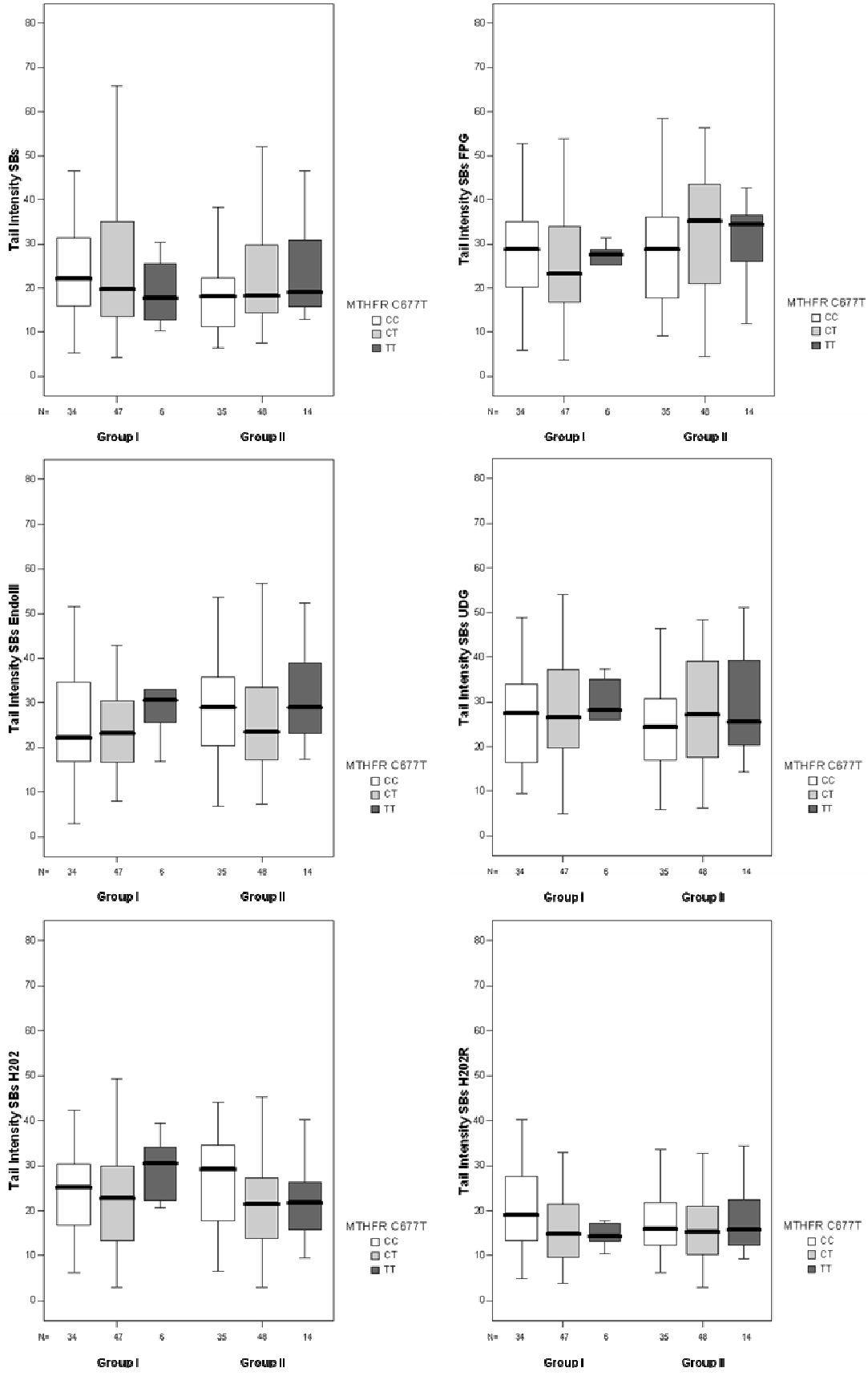

Figure 4

Influence of *XRCC1 194Thr* gene polymorphisms on DNA damage (Tail Intensity).

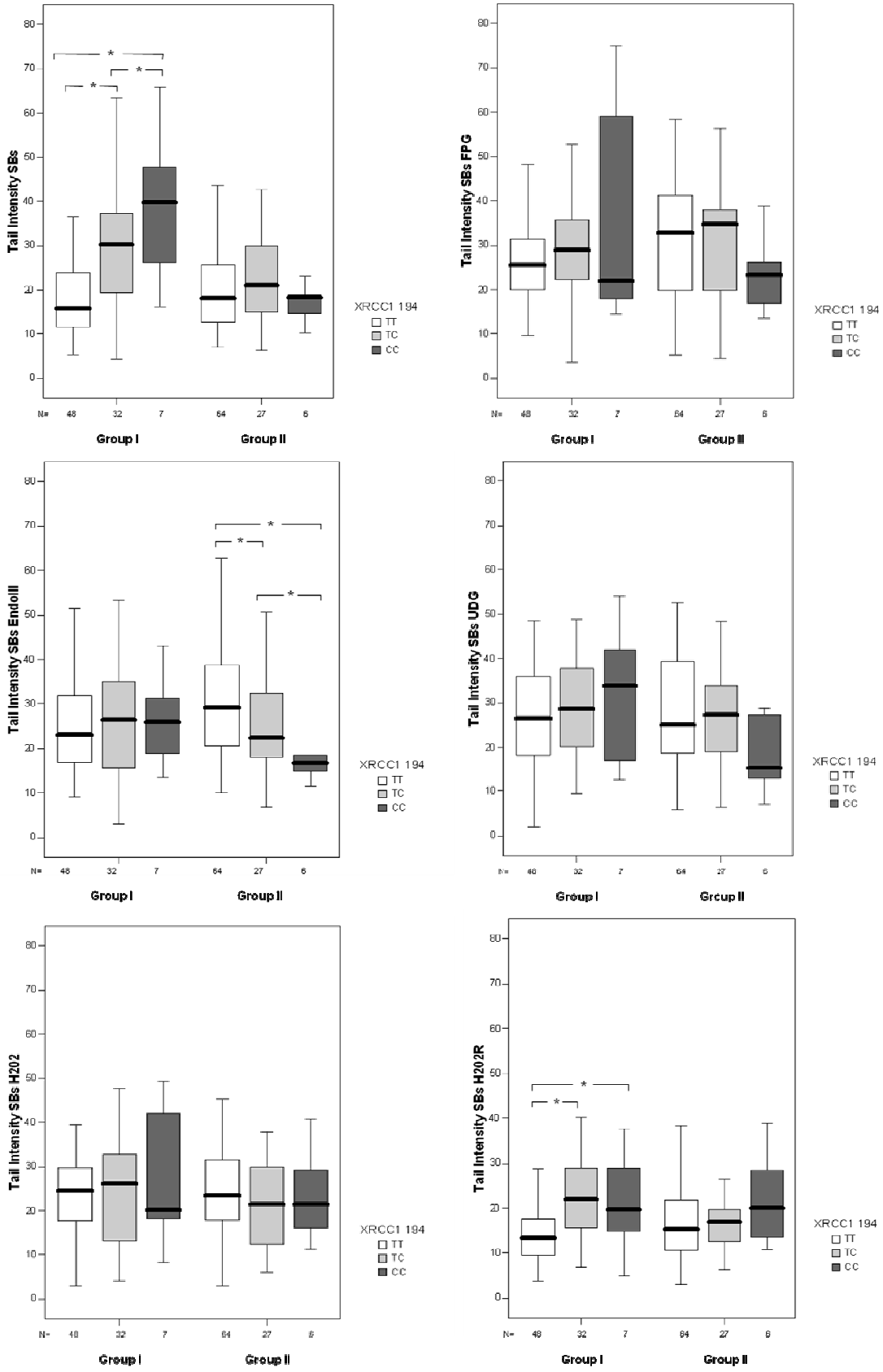

Figure 5

Influence of *XRCC1 399Gln* gene polymorphisms on DNA damage (Tail Intensity).

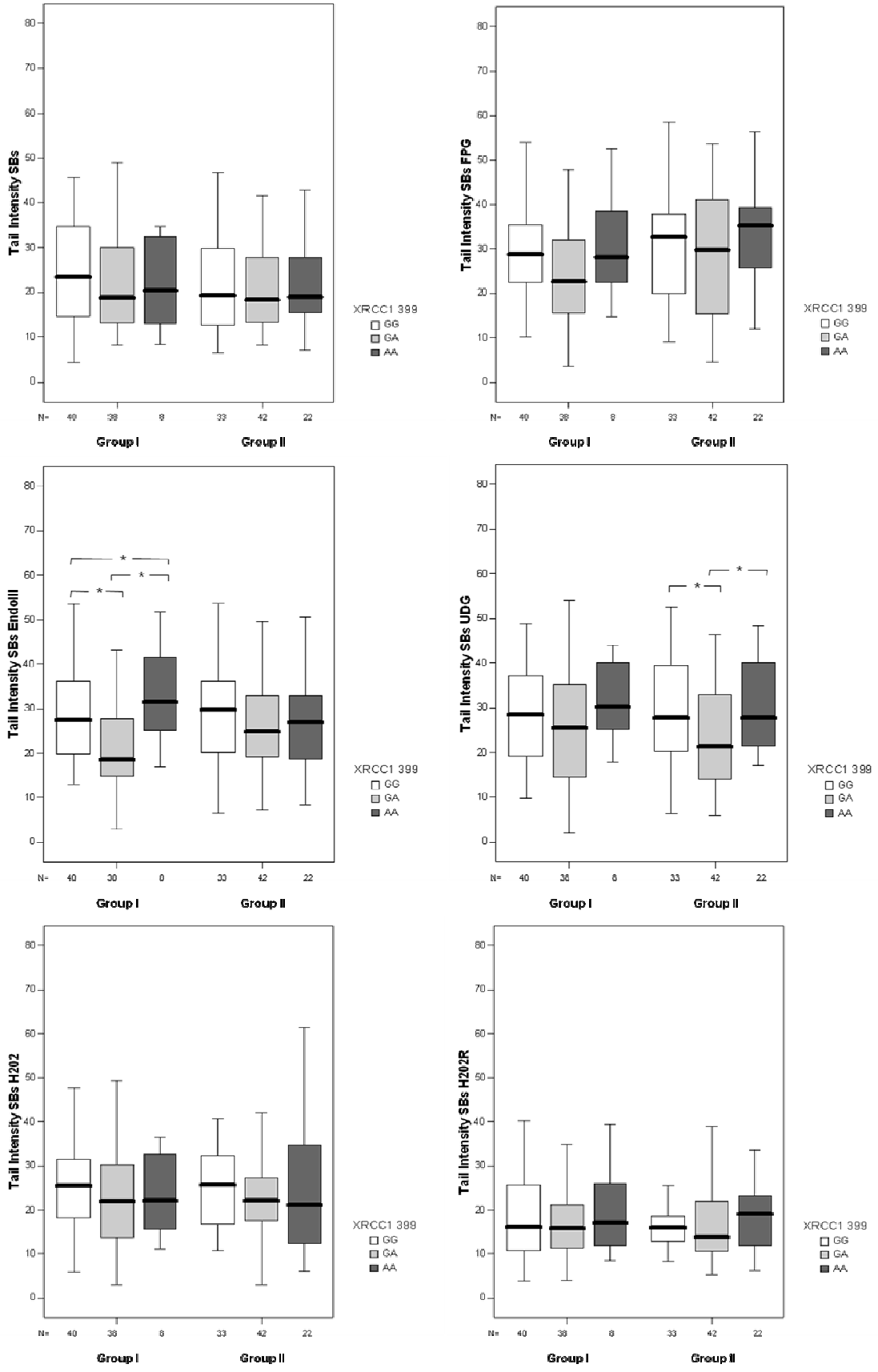

Figure 6

Conclusões Gerais

As análises de nossos resultados fornecem evidências de que uma dieta rica em grãos integrais, frutas e legumes e pobre em produtos industrializados pode proteger contra danos oxidativos no DNA. No entanto, é importante ter uma ingestão adequada de nutrientes, pois tanto a deficiência quanto o excesso de micronutrientes podem levar a um aumento de danos no DNA. Alem disso, os polimorfismos de genes de metabolismo de xenobióticos (*GSTP1*) e reparo de DNA (*XRCC1 194Trp e 399Gln*) podem modular os níveis de danos no DNA e a eficiência do sistema de reparo de DNA.

Anexos

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

EXPLICAÇÃO PARA O PACIENTE (NOVOS VOLUNTÁRIOS)

Gostaria de pedir sua contribuição para o projeto "INFLUÊNCIA DO PADRÃO ALIMENTAR SOBRE OS NÍVEIS DE DANOS OXIDATIVOS NO DNA E SOBRE OS NÍVEIS DE EXPRESSÃO GÊNICA". Sua ajuda será extremamente valiosa e nos permitirá analisar a influência da dieta e de alguns genes envolvidos no metabolismo e no conserto do DNA com o nível de quebras no DNA. Caso o(a) Sr.(a) aceite participar do estudo, serão coletados 20 ml do seu sangue, através do braço, com seringa e agulhas descartáveis e estéreis, para verificarmos as quebras do DNA do seu sangue, os tipos e níveis de expressão de vários genes, como por exemplo, *GSTM1, GSTT1, GSTP1, CYP2E1, MTHFR* e *TS* e o nível de vários micronutrientes no seu plasma sangüíneo, como por exemplo o nível de ácido fólico. Além disso, será preenchido um questionário, no qual o(a) senhor(a) nos dará várias informações sobre os seus hábitos alimentares.

Os seus dados genéticos e os resultados de exames e testes, gerados por esta pesquisa, não serão divulgados nem ficarão acessíveis a terceiros, incluindo empregadores, empresas seguradoras e instituições de ensino e não serão utilizados para propósitos judiciais (como, por exemplo, teste de paternidade), ou outros fins. Todo o seu material genético será codificado e todos os dados obtidos ou fornecidos pelo Sr. (a) serão inseridos em um banco de dados e o acesso só será permitido através de senha, que ficará sob a guarda exclusiva do Ms. Renato Paschoal Prado, responsável por esta pesquisa. O Sr. (a) terá completo acesso a todas as informações relacionadas ao seu material genético e aos seus resultados de exames e testes, gerados pelo nosso estudo.

Caso alguns dos dados obtidos se torne importante para o seu tratamento, prevenção ou cálculo de risco de alguma doença, somente se o senhor (a) desejar, estes dados lhe serão imediatamente comunicados.

Como o senhor (a) deve acompanhar pela televisão a genética e a medicina têm evoluído muito e por isso eu gostaria de pedir seu consentimento para continuar a usar o seu material genético em estudos futuros, que contribuirão mais ainda para o entendimento dos mecanismos de desenvolvimento de câncer. Quando necessitarmos utilizar o seu material genético para novos estudos, da mesma maneira que está sendo feito agora, entraremos em contato novamente para explicar o novo estudo e para solicitar permissão para utilização do seu material genético armazenado, portanto seu material genético só será utilizado se o Sr. (a) permitir.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DEPARTAMENTO DE PATOLOGIA - FMB – BOTUCATU – SP

I. Identificação do paciente ou responsável legal

Nome:		
RG:	Sexo: ☐ M ☐ F	Código (não preencher) :
Endereço:		
Cidade:		Bairro:
CEP:	Estado:	Telefone:

II. DADOS SOBRE A PESQUISA CIENTÍFICA

1. Título do Protocolo de Pesquisa: "INFLUÊNCIA DO PADRÃO ALIMENTAR SOBRE OS NÍVEIS DE DANOS OXIDATIVOS NO DNA E SOBRE OS NÍVEIS DE EXPRESSÃO GÊNICA."

2. Pesquisadores-Responsáveis: Ms. Renato Paschoal Prado e Dr. Marcelo Sady Plácido Ladeira, **DEPARTAMENTO DE PATOLOGIA**

3. Avaliação do Risco da Pesquisa: Sem risco

4 .Duração Prevista da Participação do Paciente : vinte minutos.

III. REGISTRO DAS EXPLICAÇÕES DO PESQUISADOR AO PACIENTE OU SEU REPRESENTANTE LEGAL SOBRE A PESQUISA, CONSIGNANDO:

1. Justificativa e Objetivos da pesquisa: Contribuir para o esclarecimento do papel dos padrões alimentares na manutenção da integridade do DNA

2. Procedimentos utilizados: Serão coletados: 20 ml de sangue periférico. Além disso, será preenchido um extenso questionário.

3. Desconfortos e riscos: Somente a picada para coleta de sangue. Não há riscos

4. Benefícios que poderão ser obtidos: Contribuição para o esclarecimento dos efeitos da alimentação sobre doenças degenerativas crônicas, em especial o câncer

5. Procedimentos vantajosos para o indivíduo: Avaliação do seu nível de danos no DNA.

IV - ESCLARECIMENTOS DADOS PELO PESQUISADOR SOBRE GARANTIAS DO SUJEITO DA PESQUISA:

1. Fui esclarecido sobre a garantia de ter acesso, a qualquer tempo, **aos meus dados genéticos**, às informações sobre procedimentos, riscos, benefícios, e outros assuntos relacionados com a pesquisa, inclusive para dirimir eventuais dúvidas?

[ ] Sim [ ] Não

2. Fui esclarecido de que a segurança de minha identidade será preservada, mantendo-se todas informações em caráter confidencial?

[ ] Sim [ ] Não

3. Fui esclarecido sobre a disponibilidade de assistência no HCFMUNESP, por eventuais danos á saúde, decorrentes da pesquisa?

[ ] Sim [ ] Não

4. Fui esclarecido sobre a viabilidade de indenização por eventuais danos à saúde decorrentes da pesquisa?

[ ] Sim [ ] Não

5. Fui esclarecido que não receberei qualquer remuneração financeira por participar desta pesquisa. [ ] Sim [ ] Não

6. Fui informado que terei a liberdade de retirar **meu material genético**, meu consentimento e sair desta pesquisa a qualquer momento.

[ ] Sim [ ] Não

7. Fui informado que os médicos e pesquisadores que participam deste projeto de pesquisa estarão à minha disposição para esclarecimento de qualquer questão relacionado à pesquisa. [ ] Sim [ ] Não

V. INFORMAÇÕES DE NOMES, ENDEREÇOS E TELEFONES DOS RESPONSÁVEIS PELO ACOMPANHAMENTO DA PESQUISA, PARA

CONTATO EM CASO DE INTERCORRÊNCIAS CLÍNICAS E REAÇÕES ADVERSAS.

Nome: Ms Renato Paschoal Prado.

Endereço: DEPARTAMENTO DE PATOLOGIA– Faculdade de Medicina de Botucatu, UNESP, Rubião Júnior S/N, Botucatu – SP.

Telefone: (14) 3811-7263.

VI. CONSENTIMENTO PÓS-INFORMADO

Eu, _____ abaixo assinado, declaro que fui esclarecido sobre o objetivo do presente estudo, sobre eventuais desconfortos que poderei sofrer, assim como sobre os benefícios que podem resultar do estudo. Concordo, portanto, em participar, na qualidade de voluntário, do referido Projeto de Pesquisa, sob livre e espontânea vontade e permito a utilização do meu material genético em estudos futuros.

_____, _____ de _____ de _____

_____

_____

Paciente Pesquisador

Questionário de Frequência Alimentar

Data da entrevista ____/____/_____ Hora de Início: _____
Nome do entrevistador: _____
Nome do voluntário: _____Sexo( )F ( )M
Idade atual: _____ Data de nascimento: ____/____/_____

1. Você mudou seus hábitos alimentares recentemente ou está fazendo dieta para emagrecer ou por qualquer outro motivo?
   (1) Não
   (2) Sim, para perda de peso
   (3) Sim, por orientação médica
   (4) Sim, para dieta vegetariana ou reduçao do consumo de carne
   (5) Sim, para redução de sal
   (6) Sim, para redução de colesterol
   (7) Sim, para ganho de peso
   Outro motivo: _____

2. Você está tomando algo para suplementar sua dieta (vitaminas, minerais e outros produtos)?
   (1) Não(2) Sim, regularmente (3) Sim, mas não regularmente

3. Se a resposta da pergunta anterior for sim, por favor preencher o quadro abaixo:

Suplemento	Marca Comercial	Dose	Freqüência

4. As questões seguintes relacionam-se ao seu hábito alimentar usual no período de um ano. Para cada quadro abaixo responda, por favor, a freqüência que melhor descreva quantas vezes você costuma comer cada item e a respectiva unidade de tempo (se por dia, por semana, por mês ou no ano). Depois responda qual a sua porção individual usual em relação à porção média indicada. Escolha somente um círculo para cada coluna. Se você não come ou raramente come um determinado item, preencha o círcula da primeira coluna (N= nunca come). Não deixe itens em branco.

Grupo de alimentos	Com que freqüência você costuma comer?		Qual o tamanho de sua porção em relação à porção média?	
	Quantas vezes você come:	Unidade	Porção média (M)	Sua porção
Alimentos e preparações	Número de vezes: 1,2,3, etc. (N= nunca ou raramente come)	D= por dia S= por semana M= por mês A= por ano	Porção média de referência	P= menor que a porção média M= igual à porção média G= maior que a porção média E= bem maior que a porção média

Sopas e massas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Sopas (de legumes, canja, creme, etc)	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 concha média (150g)	P O	M O	G O	E O
Salgados fritos (pastel, coxinha, risólis, bolinho)	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade grande (80g)	P O	M O	G O	E O
Salgados assados (esfiha, bauruzinho, torta)	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 unidades ou 2 pedaços médios (140g)	P O	M O	G O	E O
Macarrão com molho sem carne	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 prato raso (200g)	P O	M O	G O	E O
Macarrão com molho com carne, lasanha, nhoque	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 escumadeira ou 1 pedaço pequeno (110g)	P O	M O	G O	E O
Pizza, panqueca	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias pequenas ou 2 unidades (180g)	P O	M O	G O	E O
Polenta cozida ou frita	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa ou 2 fatias pequenas (70g)	P O	M O	G O	E O
Cereais integrais	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Aveia, quinoa, linhaça, trigo	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 colheres de sopa (40g)	P O	M O	G O	E O
arroz	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 escumadeiras médias (120g)	P O	M O	G O	E O
Pães e biscoitos	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Pão francês, pão de forma, pão doce, torrada	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade ou 2 fatias (50g)	P O	M O	G O	E O
Pão de forma integral	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias (50g)	P O	M O	G O	E O
Biscoito sem recheio	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	4 unidades (24g)	P O	M O	G O	E O
Biscoito recheado, waffer, amanteigado	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 unidades (41g)	P O	M O	G O	E O
Pães e biscoitos	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Bolo simples	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (60g)	P O	M O	G O	E O
Bolo recheado	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (60g)	P O	M O	G O	E O
Hortaliças/verduras e frutas verdes	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Alface	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 folhas médias (30g)	P O	M O	G O	E O
Almeirão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 folhas médias (30g)	P O	M O	G O	E O
Agrião	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 prato de sobremesa (38g)	P O	M O	G O	E O
Brócolis	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 ramo ou 2 colheres de sopa (30g)	P O	M O	G O	E O
Chicória crua	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 prato de sobremesa (38g)	P O	M O	G O	E O
Chicória cozida	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (30g)	P O	M O	G O	E O
Couve manteiga crua	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 prato de sobremesa (38g)	P O	M O	G O	E O
Couve manteiga	N	1	2	3	4	5	6	7	8	9	10	D	S	M	A	1 colher de servir	P	M	G	E

cozida	O O O O O O O O O O O	O O O O	(30g)	O O O O
Chuchu	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Mostarda	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	3 folhas médias (30g)	P M G E O O O O
Rúcula	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 prato de sobremesa (38g)	P M G E O O O O
Espinafre	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de servir (30g)	P M G E O O O O
Pepino	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Pimentão	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Abobrinha	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Quiabo	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Abacate	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 colheres de sopa cheia (90g)	P M G E O O O O
Azeitona	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	6 unidades (25g)	P M G E O O O O
Figo	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (50g)	P M G E O O O O
Kiwi	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (40g)	P M G E O O O O
Verduras/ Hortaliças e frutas verdes	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Uva	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	10 gomos (80g)	P M G E O O O O
Jiló	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 colheres de sopa (32g)	P M G E O O O O
Verduras/Hortaliças amarelo-alaranjados	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Abóbora	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa cheia (30g)	P M G E O O O O
Cenoura	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 colher de sopa (30g)	P M G E O O O O
Mamão formosa	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 fatia média (160g)	P M G E O O O O
Mamão papaya	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ unidade média (160g)	P M G E O O O O
Manga	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (120g)	P M G E O O O O
Laranja	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (180g)	P M G E O O O O
Mexerica	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (180g)	P M G E O O O O
Tangerina	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (180g)	P M G E O O O O
Abacaxi	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 fatia grande (180g)	P M G E O O O O
Pêssego	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (150g)	P M G E O O O O
Ameixa	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (70g)	P M G E O O O O
Cajamanga	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 unidade média (150g)	P M G E O O O O

Verduras/ Hortaliças e frutas vermelhas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Tomate	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 fatias médias (40g)	P O	M O	G O	E O
Molho de tomate caseiro/lata/caixa	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1,5 colher de sopa (25g)	P O	M O	G O	E O
Extrato de tomate	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1,5 colher de sopa (25g)	P O	M O	G O	E O
Vinagrete	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 colheres de sopa (45g)	P O	M O	G O	E O
Rabanete	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de sopa cheia (30g)	P O	M O	G O	E O
Acerola	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	10 unidades (50g)	P O	M O	G O	E O
Caqui	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (120g)	P O	M O	G O	E O
Goiaba	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade grande (225g)	P O	M O	G O	E O
Pitanga	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	10 unidades (50g)	P O	M O	G O	E O
Melancia	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (150g)	P O	M O	G O	E O
Morango	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	7 unidades médias (84g)	P O	M O	G O	E O
Hortaliças/verduras vermelho-arroxeadas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Beterraba	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de sopa cheia (30g)	P O	M O	G O	E O
Repolho	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Berinjela	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de sopa cheia (30g)	P O	M O	G O	E O
Verduras/ Hortaliças e frutas branco-amareladas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Couve-flor	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 ramo (30g)	P O	M O	G O	E O
Acelga crua	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 prato de sobremesa (38g)	P O	M O	G O	E O
Acelga cozida	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (30g)	P O	M O	G O	E O
Repolho	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Melão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (150g)	P O	M O	G O	E O
Maçã	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (110g)	P O	M O	G O	E O
Banana nanica	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (100g)	P O	M O	G O	E O
Banana prata	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (86g)	P O	M O	G O	E O
Banana maçã	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (75g)	P O	M O	G O	E O
Pêra	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (110g)	P O	M O	G O	E O
Jabuticaba	N	1	2	3	4	5	6	7	8	9	10	D	S	M	A	10 unidades (70g)	P	M	G	E

	O O O O O O O O O O O	O O O O		O O O O
Tubérculos	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Mandioca cozida	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 escumadeira cheia (90g)	P M G E O O O O
Mandioca frita	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 colheres de servir cheias (100g)	P M G E O O O O
Batata frita	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 colheres de servir cheias (100g)	P M G E O O O O
Batata cozida	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 escumadeira cheia (90g)	P M G E O O O O
Batata doce frita	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 colheres de servir cheias (100g)	P M G E O O O O
Batata doce cozida	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 escumadeira cheia (90g)	P M G E O O O O
Tubérculos	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Farinha de mandioca, farofa, cuscuz, tapioca	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	3 colheres de sopa (40g)	P M G E O O O O
Bebidas	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Suco de tomate	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de laranja	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de limão	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de goiaba	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de melancia	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de melão	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de acerola	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de maracujá	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de abacaxi	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de uva	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de Pêssego	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de Cajú	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Suco natural de morango	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	½ copo americano (80ml)	P M G E O O O O
Cerveja	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 latas (700ml)	P M G E O O O O
Vinho	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 taça pequena (50ml)	P M G E O O O O
Café ( ) c/ açúcar ( ) s/ açúcar	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 xícaras de café (90ml)	P M G E O O O O
Chá ( ) c/ açúcar ( ) s/ açúcar	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	2 xícaras de café (90ml)	P M G E O O O O
Refrigerantes	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 copo de requeijão (240ml)	P M G E O O O O
Suco industrializado	N 1 2 3 4 5 6 7 8 9 10 O O O O O O O O O O O	D S M A O O O O	1 copo de requeijão (240ml)	P M G E O O O O

Leite e derivados	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Leite ( ) integral ( ) desnatado ( ) Semi-desn.	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	½ copo de requeijão (125ml)	P O	M O	G O	E O
Leite e derivados	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Leite fermentado	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 pote pequeno (90ml)	P O	M O	G O	E O
Iogurte ( ) integral ( ) desnat. ( ) semi-d.	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade pequena (140g)	P O	M O	G O	E O
Queijo amarelos	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1,5 fatias grossas (30g)	P O	M O	G O	E O
Queijos brancos (ricota, minas)	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (30g)	P O	M O	G O	E O
Requeijão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 pontas de faca (15g)	P O	M O	G O	E O
Manteiga( ) comum ( )light	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 pontas de faca (15g)	P O	M O	G O	E O
Margarina( )comum ( )light	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 pontas de faca (15g)	P O	M O	G O	E O
Leguminosas e ovos	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Amendoim	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 punhados (60g)	P O	M O	G O	E O
Feijão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 concha média (86g)	P O	M O	G O	E O
Lentilha	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (35g)	P O	M O	G O	E O
Grão-de-bico	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (35g)	P O	M O	G O	E O
Guandu	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (35g)	P O	M O	G O	E O
Vagem	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de sopa cheia (30g)	P O	M O	G O	E O
Soja	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (35g)	P O	M O	G O	E O
Ervilha	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de servir (35g)	P O	M O	G O	E O
Ovo (cozido, frito)	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade (50g)	P O	M O	G O	E O
Carnes	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Carne bovina	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 bife média ou 2 pedaços (100g)	P O	M O	G O	E O
Hambúrguer, nuggets, almôdega	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade média (60g)	P O	M O	G O	E O
Frango( ) com pele ( ) sem pele	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 pedaço ou 1 filé pequeno (60g)	P O	M O	G O	E O
Carne suína	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fatia média (100g)	P O	M O	G O	E O
Vísceras	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Fígado, coração, língua, estômago, etc.	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 bife médio ou 2 pedaços (100g)	P O	M O	G O	E O

Pescados, frutos do mar	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Bacalhau, cação, salmão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 filé pequeno ou 1 posta pequena (100g)	P O	M O	G O	E O
Lambari, merluza, pintado	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 filé pequeno ou 1 posta pequena (100g)	P O	M O	G O	E O
Sardinha, atum	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 filé pequeno ou 1 posta pequena (100g)	P O	M O	G O	E O
Embutidos, processados, defumados	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Presunto	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias médias (30g)	P O	M O	G O	E O
Salame	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	4 fatias (30g)	P O	M O	G O	E O
Mortadela	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias médias (30g)	P O	M O	G O	E O
Lingüiça	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 gomo médio (60g)	P O	M O	G O	E O
Peito de perú	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias médias (30g)	P O	M O	G O	E O
Salsicha	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 fatias médias (30g)	P O	M O	G O	E O
Bacon	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 pedaços pequenos (40g)	P O	M O	G O	E O
Carne seca, carne de sol	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 pedaços pequenos (40g)	P O	M O	G O	E O
Oleaginosas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Amêndoa	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Avelã	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Castanha	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Castanha-do-pará	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Noz	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (30g)	P O	M O	G O	E O
Óleos	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Soja, girassol, canola, milho	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fio (4ml)	P O	M O	G O	E O
Azeite	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fio (4ml)	P O	M O	G O	E O
Temperos naturais	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Salsa, cebolinha, tomilho, louro, orégano, alecrim, coentro, manjericão	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de sopa (3,8g)	P O	M O	G O	E O
Doces e sobremesas	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Açúcar, mel, geléia	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	½ coher de sopa (6g)	P O	M O	G O	E O
Chocolate, bombom, brigadeiro	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 barra pequena (25g)	P O	M O	G O	E O
Achocolatado em pó (adicionado ao	N O	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (25g)	P O	M O	G O	E O

leite)																				
Sobremesas, doces, tortas e pudins	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 pedaço ou 1 fatia média (60g)	P O	M O	G O	E O
Sorvetes cremosos	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 bola grande (70g)	P O	M O	G O	E O
Doce de frutas (calda/barra)	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher grande ou 2 unidades (70g)	P O	M O	G O	E O
Outros	Quantas vezes você come											Unidade				Porção média (M)	Sua porção			
Glutamato monossódico (ex: sazon, ajinomoto)	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 pitada (0,35g)	P O	M O	G O	E O
Adoçante	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	3 gotas	P O	M O	G O	E O
Balas ou chicletes	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 unidade	P O	M O	G O	E O
Enlatados	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	2 colheres de sopa (60g)	P O	M O	G O	E O
Salgadinhos industrializados	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 pacote (30g)	P O	M O	G O	E O
Maionese	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de chá (4g)	P O	M O	G O	E O
Mostarda	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 colher de chá (4g)	P O	M O	G O	E O
Shoyu	NO	1 O	2 O	3 O	4 O	5 O	6 O	7 O	8 O	9 O	10 O	D O	S O	M O	A O	1 fio (5ml)	P O	M O	G O	E O