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**INFLUÊNCIA DO PADRÃO ALIMENTAR SOBRE OS NÍVEIS DE  
DANOS OXIDATIVOS NO DNA EM LINFÓCITOS DE SANGUE  
PERIFÉRICO**

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## RESUMO

O alto índice de neoplasias observado atualmente pode ser reflexo das mudanças ocorridas nos hábitos alimentares. O baixo consumo de frutas e vegetais, ricos em micronutrientes que atuam como antioxidantes e cofatores enzimáticos nas reações de síntese, reparo e manutenção da metilação do DNA; associado a alta ingestão de aditivos químicos na alimentação com possível ação genotóxica podem estar relacionados à eventual instabilidade genômica, favorecendo assim o desenvolvimento do câncer. O presente estudo visou avaliar a possível influência da alimentação sobre o nível de danos oxidativos, incorporação de uracila no DNA e a eficiência do sistema de reparo de DNA em linfócitos de sangue periférico de dois grupos de indivíduos com diferentes padrões alimentares. Grupo I: 49 indivíduos com alimentação rica em produtos orgânicos, grãos integrais, frutas e vegetais e pobre em produtos industrializados; Grupo II: 56 indivíduos que adotam uma alimentação rica em produtos industrializados e pobre em frutas e vegetais. Ambos os grupos constituídos de voluntários não fumantes, não etilistas e não usuários de drogas. Utilizou-se o ensaio cometa para análise de danos oxidativos em purinas e pirimidinas, nível de uracilas incorporadas no DNA e eficiência do sistema de reparo de DNA. A análise dos níveis de  $\beta$ -caroteno e licopeno no soro foi realizada por cromatografia de alta performance (HPLC). Os indivíduos do grupo I apresentaram menores níveis de purinas e pirimidinas oxidadas ( $p < 0,05$ ), menores níveis de danos induzidos pela  $H_2O_2$  ( $p < 0,05$ ) do que os indivíduos do grupo II. Não houve comprometimento da eficiência do sistema reparo de DNA em ambos os grupos ( $p < 0,05$ ). Os indivíduos do grupo I apresentaram maiores níveis de  $\beta$ -caroteno e licopeno do que os indivíduos do grupo II. Os indivíduos do grupo II apresentaram uma correlação negativa entre nível de quebras de fita dupla e simples de DNA (QFs) e  $\beta$ -caroteno ( $r = -0,349$ ,  $p < 0,04$ ) e entre uracilas incorporadas e  $\beta$ -caroteno ( $r = -0,369$ ,  $p < 0,029$ ). Os indivíduos com percentil 75 de concentração sérica de  $\beta$ -caroteno apresentaram maiores níveis de QFs ( $p = 0,044$ ), purinas oxidadas ( $p = 0,03$ ) e pirimidinas oxidadas ( $p = 0,01$ ) quando comparados aos indivíduos com



percentil 25 de  $\beta$ -caroteno, enquanto os indivíduos com percentil 75 de concentração sérica de licopeno apresentaram maior nível de purinas oxidadas quando comparadas aos indivíduos com percentil 25. Nossos resultados sugerem que a alimentação rica em produtos orgânicos, grãos integrais, frutas e vegetais e pobres em produtos industrializados pode ter efeito protetor contra danos oxidativos no DNA. Entretanto, em altas concentrações (percentil 75), tanto  $\beta$ -caroteno como licopeno podem levar ao aumento de danos no DNA.

## I. REVISÃO BIBLIOGRÁFICA

### I.1 – Considerações Iniciais

Em 2005, de um total de 58 milhões de mortes ocorridas no mundo, o câncer foi responsável por 7,6 milhões, o que representou 13% de todas as mortes (INCA, 2007). Dieta e fatores relacionados à nutrição desempenham um importante papel na carcinogênese (Watters et al., 2007).

Vários aditivos químicos alimentares têm atividade carcinogênica comprovada, 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. Muitos estudos têm demonstrado maior expectativa de vida e menor risco para o desenvolvimento de doença isquêmica do coração em indivíduos que optam por dietas ricas em vegetais (Trichopolou *et al.*, 2003). Além disso, vários estudos também têm descrito um efeito protetor de dietas ricas em frutas e vegetais em alguns tipos de câncer (IARC, 2003; Loft *et al.*, 2008). Porém, o modo de ação desses micronutrientes é complexo e ainda não compreendido totalmente (Loft *et al.*, 2008).

### I.2 – Dieta e Câncer

Vários tipos de cânceres apresentam incidências diferentes, com algumas regiões apresentando incidências até 100x maiores de determinados tipos de câncer como, por exemplo, melanoma e câncer de nasofaringe (Bingham e Riboli, 2004). A incidência de câncer de pulmão, intestino, mama, próstata e bexiga é

cerca de três vezes maior no nordeste da Europa quando comparada à incidência de alguns países africanos (Bingham e Riboli, 2004). No Brasil também existe uma distribuição heterogênea da incidência de câncer, estimando-se que em 2008 e 2009 ocorrerão 466.730 casos novos de câncer. Os tipos mais incidentes (excluindo o câncer de pele do tipo não melanoma) serão os cânceres de próstata e de pulmão, no sexo masculino, e os cânceres de mama e de colo do útero, no sexo feminino (INCA, 2007). Existem evidências que estas diferentes incidências de câncer estão altamente associadas a fatores ambientais e ao estilo de vida, com uma grande proporção desta variação relacionada à dieta (Bingham e Riboli, 2004).

Em 1981, Doll e Peto estimaram que nos EUA 35% dos casos de câncer estavam relacionados a fatores alimentares. Entretanto, estudos posteriores demonstraram que essa proporção pode variar de 10% a 80%, dependendo do tipo de câncer (Levi, 1999; Popkin, 2007).

Três constituintes/contaminantes da dieta tais como, álcool, aflatoxina e alimentos salgados, juntamente com a obesidade e a baixa ingestão de frutas e vegetais (La Vecchia *et al.*, 2003), já foram claramente associados ao aumento da incidência de câncer em seres humanos (Montesano e Hall, 2001). Ferguson (2002) mostrou que um grupo de produtos químicos conhecidos como aminas heterocíclicas e hidrocarbonetos policíclicos aromáticos, produzidos durante o preparo da carne em altas temperaturas, e aditivos químicos utilizados para conservação da carne (componentes N-nitrosos), podem levar ao câncer de cólon e de próstata. Estes produtos químicos foram descritos como mutagênicos em bactérias e carcinogênicos em roedores (Wasson *et al.*, 2008). Além disso, alguns

estudos epidemiológicos têm sugerido que o consumo de produtos gerados no preparo de carnes grelhadas também pode ser carcinogênico para os seres humanos (Pfau et al., 1999).

Por outro lado, vários estudos epidemiológicos têm demonstrado que indivíduos que consomem grande quantidade de frutas e vegetais ricos em micronutrientes, tais como vitamina C, vitamina E, carotenóides e flavonóides, apresentam menor incidência de câncer de pulmão, estômago, mama, cólon, faringe e fígado (Moller e Loft, 2002). Possivelmente a dieta reduz o risco de câncer devido a atividade antioxidante desses micronutrientes, que diminuem o impacto negativo das espécies reativas de oxigênio (ROS) (Watters et al., 2007; Loft et al., 2008). Essas ROS são geradas no corpo através do metabolismo normal ou devido a exposição a agentes químicos que sofrem a ação de enzimas envolvidas no processo de biometabolismo levando a geração de ROS, as quais podem induzir diversos tipos de danos no DNA, os quais se não forem adequadamente reparados, podem levar a mutações, que podem constituir importante passo da carcinogênese (Wasson et al., 2008). Portanto, os nutrientes antioxidantes podem reduzir o risco de câncer inibindo danos oxidativos no DNA (Cerutti, 1994; Cozzi *et al.*, 1997; Pool-Zobel *et al.*, 1997), sendo considerados como agentes potencialmente quimiopreventivos (Bonne *et al.*, 1990).

Vários micronutrientes atuam como cofatores enzimáticos nas reações de manutenção, reparo e metilação do DNA e no processo de apoptose (Ames e Wakimoto, 2002). Variações na ingestão de micronutrientes podem estar relacionadas a eventual instabilidade genômica detectada pelo aumento na incidência de biomarcadores como mutação de ponto, quebra cromossômica ou

ainda, por interferir na segregação cromossômica, expressão gênica, estresse oxidativo, necrose e apoptose (Fenech & Ferguson, 2001). Segundo Fenech (2007) os danos genômicos causados por uma moderada deficiência de micronutrientes são equivalentes aos níveis de danos genômicos causados pela exposição a doses significativas de agentes genotóxicos ambientais, tais como carcinógenos químicos, radiação ultra-violeta (UVA) e ionizante. Cerca de 40 micronutrientes, entre vitaminas, minerais essenciais e outros componentes são requeridos na dieta humana, em pequenas quantidades, para um metabolismo eficiente. Entretanto, não há consenso sobre o nível de micronutrientes necessários para prevenção de danos no DNA em seres humanos (Ferguson, 2002).

Dietas ricas em micronutrientes têm sido relacionadas à prevenção de danos oxidativos no DNA, otimização do processo de apoptose (Ferguson, 2002), e ao aumento da capacidade de reparo de danos no DNA. Collins *et al.* (2003) adicionando kiwi como suplemento alimentar durante três semanas a indivíduos saudáveis verificaram um aumento no nível de antioxidantes e maior eficiência do sistema de reparo do DNA, e sugeriram que estes efeitos poderiam diminuir o risco de desenvolvimento de câncer.

A desnutrição causada por deficiência de nutrientes é principalmente um problema relacionado a condições associadas à pobreza, ignorância e à senilidade, sendo um fator importante em países em desenvolvimento. Existe uma preocupação da Organização Mundial de Saúde não só com a desnutrição protéico-calórica, mas, também, com o estado micronutricional. A carência de determinados micronutrientes (sendo os principais, o iodo, o ferro, o folato, o

cálcio e as vitaminas A e D) pode provocar uma série de doenças e seqüelas, desde a diminuição na imunidade, osteoporose, cegueira, bócio, anemia, diminuição da capacidade de aprendizado, letargia, retardo mental, até a morte. Algumas doenças que provavelmente estão relacionadas à alimentação inadequada e que apresentam grande expressão mundial merecem destaque, como por exemplo, o câncer, a osteoporose e outras doenças degenerativas (cardiovasculares, doença de Alzheimer, envelhecimento precoce) (Fenech & Ferguson, 2001). De acordo com 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, doenças cardíacas e ao desenvolvimento de câncer, especialmente do cólon e reto (Duthie, 1999). O ácido fólico e a vitamina B12 desempenham papel importante no metabolismo do DNA. Assim, em condições de deficiência de ácido fólico, a uracila é incorporada ao DNA no lugar da timina (Eto & 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 um acúmulo de sítiosapurínicos/apirimidínicos e quebras de fitas simples e duplas (Ames, 2001; Fenech, 2001), que podem levar a mutações pontuais e aberrações cromossômicas grosseiras (Blount & Ames, 1995; Blount *et al.*, 1997). Além disso, o ácido fólico, bem como a vitamina B12, são necessários para a síntese de metionina e S-adenosil metionina (SAM), agente necessário para a manutenção

dos padrões de metilação do DNA, que atua na regulação da expressão gênica e na conformação da molécula (Duthie, 1999).

Os carotenóides constituem uma família de mais de 600 membros já identificados na natureza, sendo que cerca de 50 apresentam atividade pró-vitamínica A, ou seja, podem funcionar como precursores de vitamina A em mamíferos (Olson,1989). Destes, o  $\beta$ -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). Algumas das maiores fontes de carotenóides são: cenouras e abóboras ( $\alpha$ - e  $\beta$ -caroteno). Os carotenóides são sintetizados apenas por microorganismos e plantas, sendo necessário aos humanos obtê-los através da alimentação (Talegawkar *et al.*, 2008). Estudos *in vitro*, que avaliaram os efeitos dos carotenóides sobre o processo de reparo do DNA analisaram um conjunto de células humanas, incluindo células de leucemia, onde  $\beta$ -caroteno diminuiu danos no DNA induzidos pelo ácido peroxinitroso (Hiramoto *et al.*, 1999), células de hepatoma [licopeno diminuiu lesões oxidativas (Park *et al.*, 2005)], melanócitos [(licopeno provocou uma redução de danos induzidos por UVA (Smit *et al.*, 2004)] e células de neuroblastoma [luteína e zeaxantina aumentaram danos no DNA induzidos por UVA (Santocono *et al.*, 2006)]. Existem ainda vários relatos na literatura da existência de uma relação inversa entre a ingestão e/ou níveis séricos de vitamina C, vitamina E ou carotenóides (principalmente  $\beta$ -caroteno) e 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 a combinação entre ácido ascórbico,  $\alpha$ -tocoferol e  $\beta$ -caroteno torna-se mais efetiva, visto que esses

micronutrientes podem interagir no ambiente celular e potencializar a defesa antioxidante (Niki *et al.*,1995) protegendo contra dano oxidativos no DNA (Anderson,1996; Cozzi *et al.*,1997; Pool-Zobel *et al.*,1997). Porém, quando administrado em excesso ou em quantidades muito acima daquelas normalmente ingeridas pelas populações saudáveis,o  $\beta$ -caroteno pode agir como pró-oxidante, ocasionando efeitos tóxicos, inclusive potencializando o estado oxidativo crônico presente nos pulmões de tabagistas inveterados (Naves & Moreno,1998; Palozza,1998).

O licopeno, outro importante carotenóide, vem sendo bastante estudado devido a sua atividade antioxidante (Bramley, 2000). Trata-se de um carotenóide acíclico, composto por átomos de carbono e hidrogênio unidos por 11 duplas ligações conjugadas (Bramley, 2000). Embora seja também encontrado em algumas frutas como a goiaba, melancia e mamão papaia, o tomate é sua principal fonte (Bramley, 2000). Estudos experimentais e epidemiológicos mostraram a ação protetora do licopeno sobre doenças cardiovasculares e vários tipos de cânceres (principalmente da mama, da próstata e do cólon) (Bramley, 2000). Embora seja desconhecido o mecanismo exato pelo qual o licopeno exerça tais ações, existem as hipóteses de que o licopeno exerça atividade redutora sobre o oxigênio *singlet* (Di Mascio, 1989); proteção contra lesões de membrana causadas pelo radical dióxido de nitrogênio e peróxido de hidrogênio (Böhm *et al.*, 1995); aumento das comunicações intercelulares via *gap junctions*; inibição da proliferação celular interferindo nas sinalizações celulares de fatores de crescimento; estímulo ao metabolismo de xenobióticos e a modulação do processo inflamatório.



É importante salientar que as deficiências de vitaminas e minerais podem ser reduzidas com intervenções e investimentos relativamente pequenos em saúde pública, e por meio de estratégias que visam melhorar o acesso, a disponibilidade e o consumo de alimentos ricos em vitaminas e minerais. A ingestão ideal de um determinado micronutriente pode variar com a idade, constituição genética, estilo de vida e hábito cultural, entre outros. Mensurar o nível ideal de ingestão de micronutrientes e combater carências, será o maior desafio nas próximas décadas, uma vez que para cada enfermidade o nível ideal de ingestão do mesmo micronutriente pode variar significativamente (Ames, 2001). Assim, a ação conjunta de áreas do conhecimento como genética toxicológica, nutrição, ciência e tecnologia dos alimentos, ciência social e comportamental e ciência ambiental pode trazer importante contribuição para a melhoria da qualidade de vida da população humana, principalmente em países em desenvolvimento.

## **II – OBJETIVOS**

Devido à importância dos fatores alimentares no processo carcinogênico este estudo teve 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 correlação entre os níveis de  $\beta$ -caroteno e de licopeno 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 no DNA de sangue periférico.

**Manuscrito I**

# **Influence of Diet on Oxidative DNA Damage, Uracil Misincorporation and DNA Repair Capability**

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Footnotes:

## Abstract

The contribution of diet to cancer ranges from 10% to 80%. The low ingestion of antioxidants and enzymatic cofactors involved in DNA-repair and methylation reactions and the high ingestion of chemical additives present in the modern diet, associated with genetic factors, could lead to genomic instability and the hypomethylation of proto-oncogenes, thus contributing to cancer development. The present study evaluated the influence of diet on the level of oxidative DNA damage, misincorporated uracil and DNA repair capability in peripheral blood lymphocytes from two groups of individuals with antagonist diets: 1) 49 healthy individuals with a diet rich in organic products, whole grains, fruit and vegetables and poor in industrialized products (Group I); 2) 56 healthy individuals with diet rich in industrialized products and poor in fruit and vegetables (Group II). Oxidative DNA damage, uracil incorporation and DNA repair capability were assessed by the comet assay. The individuals in Group I presented lower levels of oxidative DNA damage (oxidized purines and pyrimidines) and lower levels of DNA damage induced by in-vitro treatment with  $H_2O_2$  than did individuals in Group II. The analysis of our results suggests that a diet rich in organic products, integral grains, fruit and vegetables and poor in industrialized products can protect against oxidative DNA damage and DNA damage induced by hydrogen peroxide ( $H_2O_2$ ).

## 1. Introduction

It is estimated that 10% to 80% of cancer cases are related to dietary factors [1], and many chemical food additives have already been proven to be carcinogenic. However, not only the increased intake of such products, but also decreased consumption of natural foods is a factor that contributes to the emergence of modern-life diseases. Many studies have shown that three diet constituents / contaminants, namely alcohol, aflatoxin and salted foods, together with obesity and low intake of fruits and vegetables [2], are clearly associated with the increased incidence of cancer in humans [3]. Currently, high cancer rates can be seen as reflection of changes in eating habits. Diet certainly plays an important role in preventing cancer, but the mechanisms involved are still not clear [4].

Several epidemiological studies have shown that individuals who consume large amounts of fruits and vegetables rich in micronutrients with antioxidants properties, such as vitamin C, vitamin E, carotenoids and flavonoids, show a lower incidence of lung, stomach, breast, colon, liver and pharynx cancer [5]. These micronutrients can act as enzymatic cofactors in DNA maintenance, repair and methylation and in the process of apoptosis [6]. Variations in the intake of micronutrients may be related to genomic instability detected by the increased incidence of biomarkers, such as point mutation and chromosomal breakage, or by interfering in chromosomal segregation, gene expression, oxidative stress, necrosis and apoptosis [7]. According to Fenech [8], genome damage caused by moderate micronutrient deficiency 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.

Approximately 40 micronutrients, such as vitamins, essential minerals and other components are required in small quantities in the human diet for efficient metabolism. However, there is no consensus on the level of micronutrients necessary to prevent DNA damage in humans [9]. The fundamental objective of these areas is to 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 optimized for genome health maintenance [8].

According to Ames [10], the deficiency of micronutrients, such as folic acid, vitamins B12, B6, C and E, niacin, iron and zinc can mimic the effect of radiation or chemical agents in the induction of DNA damage. The deficiency of folic acid in humans has been associated with megaloblastic anemia, defects of the neural tube closure in newborns, heart disease and cancer development, especially of in colon and rectum [11]. In 2005, Fenech et al. [12] observed that chromosomal damage in a culture of human lymphocytes caused by the reduction in the concentration of folic acid from 120 nmol/L to 12 nmol/L was equivalent to that induced by acute exposure to ionizing radiation (0.2 Gy), a dose of radiation which is approximately tenfold the annual exposure safety threshold for radiation workers. Folic acid and vitamin B12 play an important role in DNA metabolism. Thus, in conditions of folic-acid deficiency, uracil is incorporated into DNA in place of thymine [13]. There is evidence showing that the excessive incorporation of uracil

in DNA not only generates point mutations, but can also cause single- and double-strand breaks, as well as lead to the formation of micronuclei [14, 15].

Another important aspect is defense against oxidative DNA damage by DNA repair. Several studies have shown how many dietary factors influence DNA metabolism and repair may act as co-factors or substrates in these essential metabolic pathways [6,7,12,16,17]. Ames and Wakimoto [6] have observed that micronutrient deficiency, such as that of Zn (involved in removing oxidized guanine) and Mg (co-factor for many DNA polymerases), may reduce enzymatic activity.

Based on the information presented, 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.

## **2. Material and Methods**

### **2.1. Subject selection**

This study was approved by the Ethics Committee for Human Research of the Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP, Brazil. Informed consent was obtained from each volunteer.

A total of 105 healthy adult volunteers (mean age  $35.6 \pm 11.4$ , ranging from 19 to 66 years), 52 males and 53 females, was studied. All of the volunteers were nonsmokers, were not abusing alcohol, were not using prescription or recreational



drugs or any vitamin or mineral supplementation. These volunteers were distributed in two groups: 1) Group I - 49 naturalistic individuals with a life style characterized by the absence of some types of stress, a bucolic life and great ingestion of organic fruits, vegetables and juice and poor consumption of industrialized foods; 2) Group II - 56 individuals with great ingestion of industrialized foods and poor ingestion of fruits and vegetables.

For evaluation of the dietary pattern, the estimated level of micronutrients ingested was calculated (data not shown), and for the group classification, a food-frequency questionnaire adapted from Cardoso and Stocco [18] was applied. This quantitative questionnaire provided data, which were analyzed by using the AvaNutri software package (Avanutri Informática Ltda, RJ, Brazil). This procedure enabled us to establish an estimate of the quantity of protective foods consumed daily by the population studied. Moreover, one dietary record was applied on the weekend, and the others two dietary records were applied during the week, but with one day spaced, to improve the calculation of the habitual dietary intake. Dietary recalls and the software were conducted by a person trained (B.F.S). To be classified in Group I, the individuals had to consume percentile 75 of organic fruits and vegetables and should less than percentile 25 of each industrialized product. The individuals in Group II were those who consumed less than percentile 25 of organic fruits and vegetables and more than the percentile 75 of industrialized products.

Only the individuals adopting the respective diet pattern for at least 10 years were selected.

## **2.2. Determination of Oxidative DNA Damage and level of uracil incorporation into DNA**

The alkaline Comet assay [19], modified with lesion-specific enzymes, was used to detect single- and double-strand breaks, labile sites (SBs), oxidized purines and pyrimidines [20] and uracil [11]. Briefly, 10  $\mu\text{L}$  of the isolated lymphocyte suspension [21] ( $\cong 2 \times 10^4$  cells) were embedded into 0.5% low melting point agarose (Sigma) and spread on agarose-precoated microscope slides. 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°C. After lysing, the slides were washed three times in enzyme buffer (Trevigen, Gaithersburg, MD) and then incubated at 37°C for 45 min with 100  $\mu\text{L}$  of endonuclease III (Endo III - 1:1000; New England Biolabs Inc), 100  $\mu\text{L}$  of formamidopyrimidine-DNA glycosylase (FPG - 1:1000; New England Biolabs Inc), 100  $\mu\text{L}$  of uracil-DNA glycosylase (UDG - 1:1000; New England Biolabs Inc) or with enzyme buffer only. Endo III recognizes oxidized pyrimidines (SBs EndoIII), while FPG identifies oxidized purines (SBs FPG), especially 8-oxo-guanine, and UDG recognizes uracil (SBs UDG). Enzyme buffer only is used to identify SBs. Subsequently the cells were exposed to alkali buffer (1mM EDTA and 300 mM NaOH, pH  $\cong$  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 and 300 mA. After electrophoresis, the slides were neutralized (0.4 M Tris, pH 7.5), stained with 50  $\mu\text{L}$  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).

One hundred randomly selected cells (50 from each of two replicate slides) were evaluated from each sample and the mean of the tail intensity (%DNA Tail) was determined. Tail Intensity, according to Comet Assay II – Perceptive instruments, is defined as “the sum of all intensity values in the tail region minus those which are part of the mirrored head region. The value is also expressed as a percentage of the total Comet or cell intensity”.

### **2.3. DNA Repair Capability**

To analyze the influence of dietary patterns on the DNA repair capability, 200  $\mu\text{l}$  of the freshly isolated lymphocytes were treated with 100  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  (100  $\mu\text{M}$ ), for 30 minutes, in ice, and analysis of the level of damage was evaluated. Then, after two washes in PBS to remove  $\text{H}_2\text{O}_2$ , 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. Log transformations were applied to DNA damage distributions to help meet normality distribution assumptions. The statistical analysis consisted of applying the t Student test to compare differences between the groups. To evaluate the influence of sex and age DNA damage levels, the Turkey test was applied. The index of significance adopted was 5%.

### 3. Results

DNA damage, as single- and double-strand breaks and apurinic/aprimidinic sites (SBs), can be evaluated by the comet formed under standard conditions. Endogenous formation of oxidized pyrimidines and purines are detected by enzymes endonuclease III (EndoIII) and formamidopyrimidine DNA glycosylase (FPG), respectively. The results showed no significant differences in the levels of SBs and misincorporated uracil in DNA between the two groups. However, the volunteers in Group I presented a lower level of oxidized purines and pyrimidines ( $p < 0.05$ ) than individuals in Group II (Figure 1).

Regarding DNA repair capacity, no difference was detected between the two groups, although the individuals in Group I presented lower levels of DNA damage induced by  $H_2O_2$  (Figure 2).

There was no influence of gender and age in the levels of oxidative DNA damage and misincorporation of uracil in DNA (Figure 3 and 4, respectively).

With respect to the level of micronutrients estimated through the Food-Frequency Questionnaire and the Recall Questionnaire, the individuals in Group I presented higher levels of vitamin A, vitamin B2, vitamin B6, vitamin B12, vitamin C and folic acid than those in Group II. In addition, the individuals in Group II presented lower estimated intake level of vitamin A, vitamin B6, vitamin C and folic acid than the Recommended Dietary Allowances (RDA) (Table 1).

## 4. Discussion

The development of cancer is related to an interaction between genetic and environmental factors, such as cigarette smoking, urban and industrial air pollution and dietary factors [22]. Several studies have demonstrated that fruit, vegetables, olive oil, red wine and cereals together with a low intake of fat from animal sources seem to be associated with lower risk for development of lung cancer, esophagus, stomach, colon and rectum cancer [1,23]. Antioxidants and flavonoids could be responsible for such protective effect. However, some of these studies have reported only the effect of isolated nutrients, and not whether there is a true relationship between them (synergistic effect) or the mechanisms involved in cancer chemoprevention, presenting only a correlation between certain nutrients/diets and carcinogenesis [24]. Therefore, better understanding of possible pathways to modulate carcinogenesis and preventive interventions during these stages become very attractive.

Several biomarkers and methodologies have been used to elucidate the role of nutrition in carcinogenesis. The Comet assay is technique widely used for detecting genotoxicity. This assay has several advantages, as it has shown to be economical, simple and fast and because, requires small number of cells [24] and provides information on antioxidant status [25]. To ensure the correct analysis of the genotoxic effects induced by dietary factors, we adopted amendments to the protocol originally described by Singh et al. [20], which also allowed the detection of specific types of DNA damage, such as oxidative damage and uracil

misincorporation besides DNA breaks induced after exposure of intact cells to ROS (reactive oxygen species). The most common ROS is hydrogen peroxide ( $H_2O_2$ ). This compound can damage cellular proteins and lipids and form DNA adducts when present in high a concentration, and this may contribute to carcinogenic activity [26].

Although some studies have not shown any significant association between diet and DNA damage, oxidative damage to DNA and chromosomal aberrations, such as between occupationally exposed men and non-occupationally PAH-exposed workers [23] or between smokers and nonsmokers [27], in the present study we observed that the healthy individuals in Group I presented lower levels of oxidized purines and pyrimidines in peripheral-blood lymphocytes. Moreover, a lower level of DNA damage was detected when the lymphocytes from these individuals were treated with  $H_2O_2$  *in vitro*. 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. It is known that some micronutrients act as enzymatic cofactors, modulating several biological processes that are relevant for cancer, including biotransformation enzymes, oxidative damage to macro-molecules, DNA adducts, DNA repair and methylation and apoptosis [6,23]. Our results are in agreement with approximately half of the studies conducted before, when significant decrease of oxidized bases and/or protection towards ROS-mediated DNA damage were described, while only one third of studies showed a decrease in DNA migration when Alkaline Comet assay standard conditions were used [25,28]. In a study using olive oil, reduced DNA damage was observed when the

experiments were conducted in Alkaline Comet assay standard conditions and with FPG. These effects were correlated with an increase in the total capacity antioxidants (TAC) plasma [29].

Another factor that could have influenced our results is the lifestyle of individuals in Group I. Their way of living is characterized by the absence of stress of modern life, with close contact with nature and organic dietary intake without chemical additives. Several studies have reported that lifestyle and environmental exposure, such as to air pollution, diet, sunlight, and exercise influence the basal level of DNA damage in leukocytes [30].

On the other hand, the higher level of oxidative DNA damage detected in individuals in Group II could be related to many chemical additives, potentially genotoxics and carcinogenics, 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 [9]. In addition, it is known that high ingestion of calories or certain foods, such as red meat, alcoholic beverages or coffee, may cause adverse effects, for example, an increase in the DNA damage induced by heterocyclic aromatic amines (PhIP), which could be explained by changes in the activities of activation and/or detoxification enzymes [31]. Ferguson [9] has also shown that some of chemical additives used for meat preservation (N-nitrous components), heterocyclic amines and polycyclic aromatic hydrocarbons, produced during the preparation of meat at high temperatures, may lead to colon and prostate cancer.

Folate deficiency upsets the balance of DNA precursors [32]. Impaired methylation of dUMP, via depletion of methyl donor 5,10-methylene

tetrahydrofolate, leads to increased levels of dUMP, low levels of dTTP and misincorporation of uracil into DNA in place of thymidine, which is probably the major cause of spontaneous DNA damage [33]. Normal cellular processes repair DNA, but as thymidine levels are suppressed, repeated misincorporation and excision repair-mediated removal of deoxy-uridine may cause DNA double-strand breakage, chromosomal aberrations and, ultimately, malignant transformation [34]. However, our results did not show differences between the levels of misincorporated uracil. Nevertheless, based on food-frequency questionnaires, the individuals in Group I presented significantly higher levels of vitamin B6, B12 and folic acid than did individuals in Group II. Moreover, the individuals in Group II presented lower levels of vitamin A, B6, vitamin C and folate than the minimum recommended daily intake (RDA), suggesting that other micronutrients and mechanisms can also be involved, as for example polymorphisms of genes related to the process of folic acid metabolism.

By adding kiwifruit to the diet of healthy individuals as food supplement for three weeks, Collins et al. [1] observed an increased level of antioxidants in blood and increased DNA repair capability, suggesting that these effects could reduce the risk for developing cancer. However little is known about the impact of micronutrient deficiency on DNA repair [35]. In the present study, we did not find differences in DNA repair capability between the groups. Both groups did present difference in DNA repair. Nevertheless, the population studied consisted of healthy volunteers, suggesting that lower levels of micronutrients intake for individuals group II is enough to maintenance of genomic stability in healthy individuals. Moreover, the genetic polymorphisms of these volunteers were not evaluated, and



gene-diet interaction is as important as micronutrient deficiency for the efficacy of DNA repair and DNA metabolism in determining genomic stability and its consequent impact on fertility, development, cancer risk and ageing rate [35,36,37,38,39]. 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 groups as regards DNA repair efficiency.

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. Further studies on a larger number of individuals must be conducted in order to also evaluate the influence of gene polymorphisms.

## **5. Acknowledgements**

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## Legends

Table 1. Level of micronutrients ingested by each group as estimated by the Food-Frequency Questionnaire and Recall Questionnaire and Recommended Dietary Allowances (RDA)

Figure 1. Levels of DNA damage (tail intensity) expressed as SBs, SBs FPG, SBs Endo III, SBs UDG, SBs H<sub>2</sub>O<sub>2</sub> and SBs H<sub>2</sub>O<sub>2</sub>R in peripheral blood lymphocytes from Group I (n=49) and Group II (n=56) 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) and 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<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The student's 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 SBs H<sub>2</sub>O<sub>2</sub> and SBs H<sub>2</sub>O<sub>2</sub>R in peripheral blood lymphocytes of individuals in Group I (n = 49) and Group II (n=56); H<sub>2</sub>O<sub>2</sub> = strand breaks induced by treatment with H<sub>2</sub>O<sub>2</sub>; H<sub>2</sub>O<sub>2</sub>R = level of DNA damage 30 minutes after DNA repair of damage induced by treatment with H<sub>2</sub>O<sub>2</sub>. Results are expressed as mean ± SD. The student's test was used to compare differences between variables.\* p < 0.05. Individuals in both groups did



not present compromised DNA repair efficiency. Individuals in Group I presented lower levels of DNA damage induced by H<sub>2</sub>O<sub>2</sub>.

Figure 3. Influence of gender on the levels of DNA damage (tail intensity) expressed as SBS, SBs FPG, SBs Endo III and SBs UDG in peripheral blood lymphocytes of individuals in Group I (n = 49) and Group II (n=56). SBs = strand breaks; SBs FPG = sites susceptible to FPG (purines injured); SBs Endo III = sites sensitive to Endo III (oxidized pyrimidines); SBs UDG = sites of incorporated uracil. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The student's test was used to compare differences between gender into groups. There was no influence of gender on DNA damage.

Figure 4. Influence of age on the levels of DNA damage (tail intensity) expressed as SBs, SBs FPG, SBs Endo III and SBs UDG in peripheral blood lymphocytes of individuals in Group I (n = 49) and Group II (n=56). SBs = strand breaks; SBs FPG = sites susceptible to FPG (Purines oxidized); SBs Endo III = sites sensitive to Endo III (Oxidized pyrimidines); SBs UDG = sites of incorporated uracil. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The student's test was used to compare differences between age into groups. The age did not influence the DNA damage level.

Table 1.

Micronutrient	RDA	Group	Mean	SD	P
Vit. A (RE)	900	1	2593.8	1065	0.001
		2	349.9	440,7	
Vit. B2 (mg)	1.3	1	2.3	0.56	0.086
		2	1.3	0.46	
Vit. B6 (mg)	1.3	1	1.9	0,37	0.036
		2	1.0	,42	
Vit. B12 (mcg)	2.4	1	7.1	3,7	0.007
		2	3.9	2,4	
Vit. C (mg)	90	1	515.1	340	0.0001
		2	34.4	104,9	
Folic acid (mcg)	400	1	448.6	145,5	0.0001
		2	125.6	70,2	

1 RE = 1 mcg of retinol

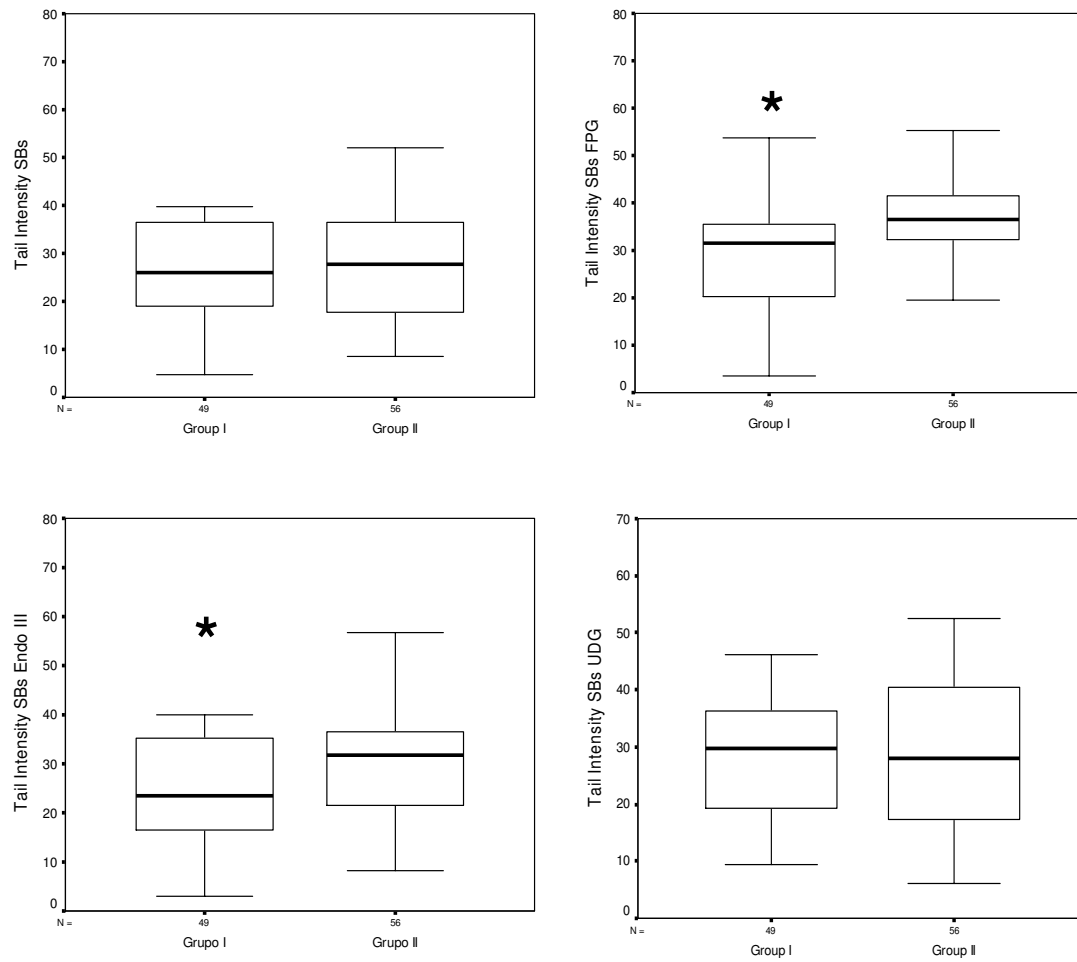


Figure 1.

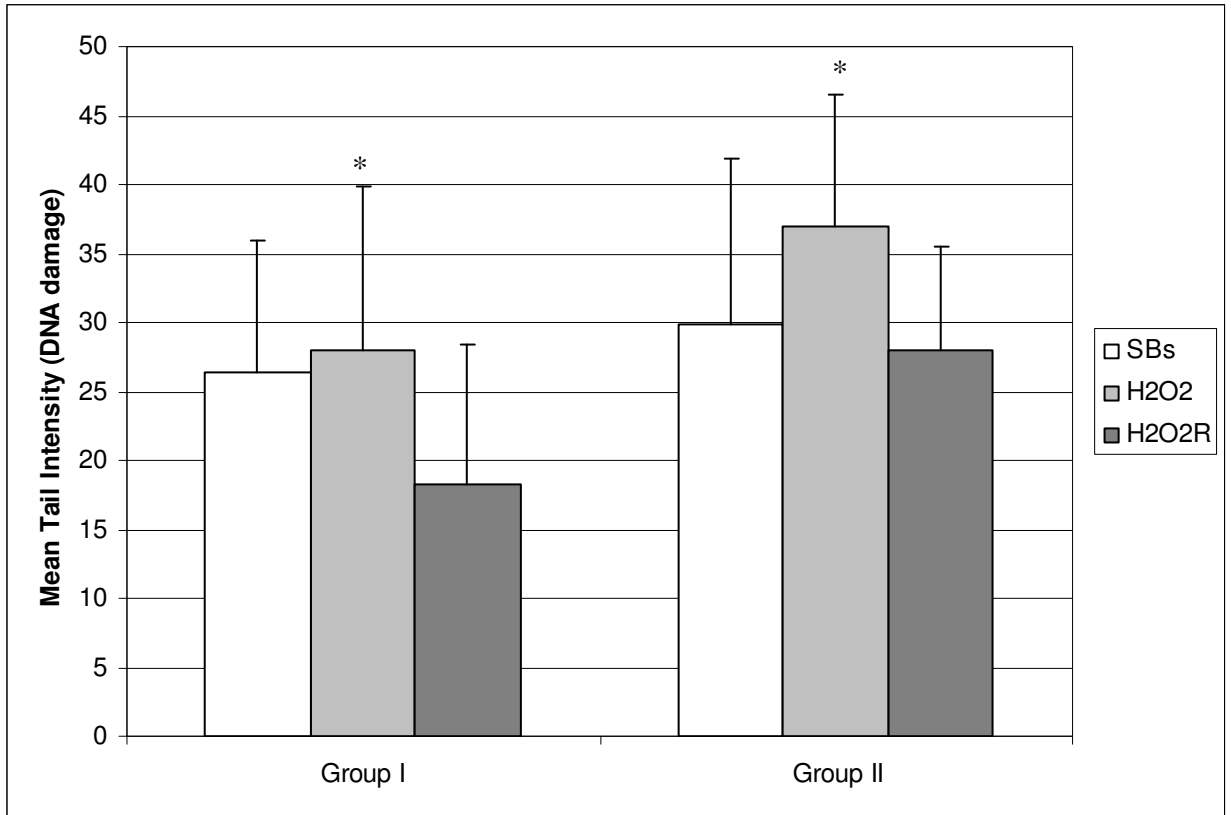


Figure 2.

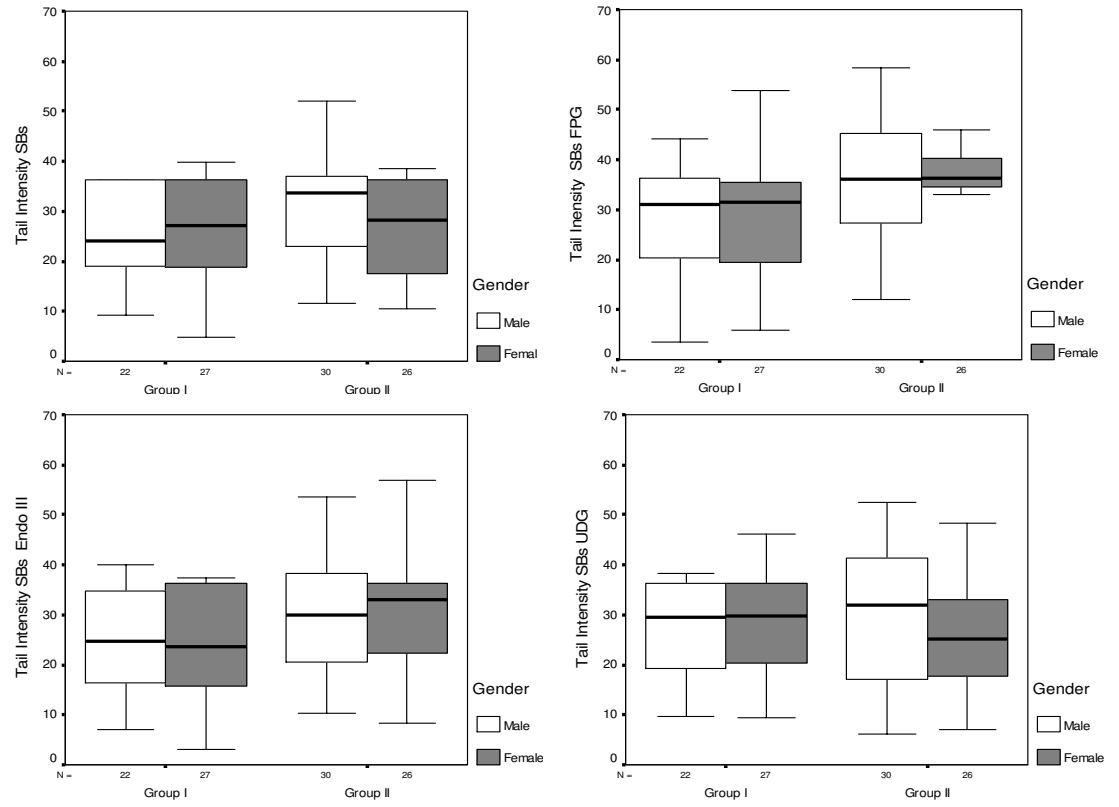


Figure 3.

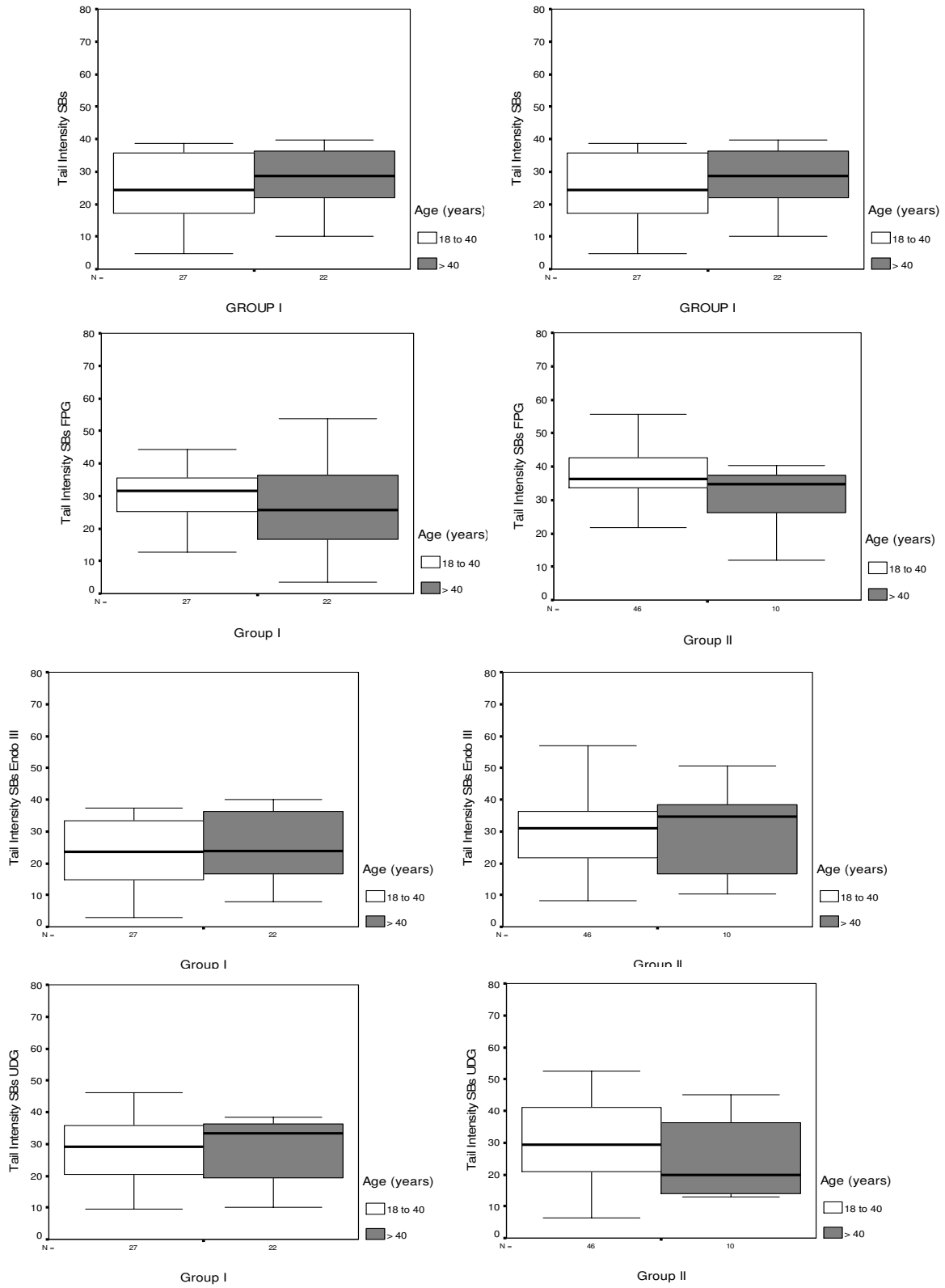


Figure 4.

**Manuscrito II**

# Influence of $\beta$ -Carotene on Oxidative DNA Damage, Misincorporated Uracil and DNA Repair Capability

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(*Running Title*): Influence of  $\beta$ -Carotene on DNA damage and repair

Key Words: Diet, oxidative DNA damage, uracil incorporation, DNA repair, comet assay,  $\beta$  - carotene

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## Abstract

The ingestion of fruits and vegetables has been correlated with low risk of developing chronic diseases, mainly with cancer. The carotenoids may be one of the components present in vegetable and fruit responsible for this lower risk. The  $\beta$ -carotene is the carotenoid more abundant in nature and is important for their antioxidant property. The present study evaluated the influence of  $\beta$ -carotene on the level of oxidative DNA damage, misincorporated uracil and DNA repair capability in peripheral blood lymphocytes from two groups of individuals with antagonist diets: 1) 49 healthy individuals with a diet rich in organic products, whole grains, fruit and vegetables and poor in industrialized products (Group I); 2) 56 healthy individuals with diet rich in industrialized products and poor in fruit and vegetables (Group II). Oxidative DNA damage, misincorporation uracil and DNA repair capability were assessed by the comet assay. The levels of  $\beta$ -carotene in the serum were measured using high-performance liquid chromatography (HPLC). The individuals of group I presented higher serum concentrations of  $\beta$ -carotene than the individuals of group II. The individuals of group II presented a negative correlation between strand breaks (SBs) and  $\beta$ -carotene ( $r = -0,349$ ,  $p < 0,04$ ) and between misincorporated uracil (SBs UDG) and  $\beta$ -carotene ( $r = -0,369$ ,  $p < 0,029$ ). The individuals that presented percentile 75 of serum concentration of  $\beta$ -carotene had higher levels of SBs ( $p = 0.044$ ), oxidised purines (SBs FPG) ( $p = 0.03$ ) and oxidised pyrimidines (SBs EndIII) ( $p = 0.01$ ) than individuals with percentile 25. The analysis of our results provide evidence that the diet rich in fruit and vegetables, rich in  $\beta$ -carotene, can be capable of protecting cells against DNA damage. Nevertheless, the high concentration of  $\beta$ -carotene can lead to increase of DNA damage.

## 1. Introduction

It's estimated that in the world, per year, around 10 million people receive the diagnosis of cancer and more than six million die due this disease [1].

Cancer presents a rate of incidence and mortality may vary from one country to another, with certain regions presenting higher incidences until 100x of certain types of cancer as, for example, melanoma and nasopharynx cancer [1]. The incidence of lung cancer, intestine, breast, prostate and bladder is approximately three times higher in the northeast of Europe than in some African countries [1]. There is evidence that these different incidences of cancer are highly associated with environmental factors and lifestyle, with a large proportion of this variation related to diet [1].

Diet certainly plays an important role in cancer, but the mechanism is still not clear [2]. It's estimated that 10% to 80% of cases of cancers are related to dietary factors [3], since many chemical additives food have proven carcinogenic activity. However, not only the increased intakes of such products, but also a decrease in the consumption of natural foods are factors that contribute to the emergence of diseases of modern life.

Several epidemiological studies have shown that individuals who consume large amounts of fruits and vegetables rich in micronutrients with properties antioxidants, such as vitamin C, vitamin E, carotenoids and flavonoids, have lower incidence of cancer of the lung, stomach, breast, colon, liver and pharynx [4]. These micronutrients act as cofactors enzymatic in the repair and methylation of DNA and in the process of apoptosis [5]. Variations in the intake of micronutrients may be related to the possible genomics instability detected by the increase in the

incidence of biomarkers as the point mutation, chromosomal breakage, or by interfering in the chromosome segregation, gene expression, oxidative stress, necrosis and apoptosis [6]. According to Fenech [7] the genome damage caused by moderate micronutrient deficiency is equivalent to the genome damage levels caused by exposure to significant doses of environmental genotoxins such as chemical carcinogens, ultra-violet radiation and ionizing radiation.

In addition, another studies have correlated the ingestion of fruit and vegetable with low risk of developing chronic diseases, mainly with cancer [8; 9], and have also proposed that carotenoids may be one of the components present in vegetable and fruits responsible for this lower risk [10; 11].

Carotenoids are family of pigmented compound synthesized by plants and microorganisms and are responsible for the colors of the nature. However, humans need to obtain them from diet [12]. The carotenoids are molecules compose for 40 carbons, which act as antioxidants to be efficient scavengers of various free radicals generated *in vitro* [13] and they have also been shown to protect low density lipoproteins (LDLs) against oxidation *in vitro* [14]. About 600 carotenoids already were isolated and characterized [15]. Besides, only about 40 are present in human diet, and about 12 carotenoids can be found in human blood and tissues in measurable concentrations [16]. Approximately 90% of the carotenoids in the diet and human body are represented by lutein, cryptoxanthin,  $\alpha$ -carotene,  $\beta$ -carotene, and lycopene [17]. These,  $\beta$ -carotene is the most abundant in nature and is found in red, green and orange plants and fruit [18; 19]. The main sources of  $\beta$ -carotene are: carrot, pumpkin, beet, papaya and mango. And can be found in smaller quantities in green vegetable as cabbage, spinach, cress and broccolis. B-carotene

is a carotenoid known as pro-vitamin A [15]. They are important for their antioxidant property and for stimulates the immune system [19; 20], since the process carcinogenic is characterized by a state oxidative chronic in the stage of promotion [21] and damage irreversible in the genetic material of the cell in the initiation phase, often due to attack of free radicals [22], especially reactive oxygen species (ROS). Consequently, the nutrients antioxidants could decrease the risk of cancer inhibiting oxidative damage in the DNA [21; 23; 24], and so considered agents potentially chemoprotective [25].

Normal metabolism generate ROS in the body, that can lead to mutations, if not repaired. These can attack various substrates such as lipids, nucleic acids and proteins [26]. Several human studies have used the comet assay as a biomarker to look at the effects of various carotenoids on DNA damage and repair [27]. In vitro studies looking at the effects of carotenoids on DNA repair have examined a range of human cell types including leukaemia cells, where  $\beta$ -carotene decreased peroxy-nitrous acid-induced damage [28]. However, some studies meet increase of the risk of lung cancer with high-dose supplementation of  $\beta$ -carotene in smokers and asbestos workers [29], probably because it can act as a pro-oxidant in the radical-rich environment of smokers' lungs [30].

The reason for carrying out this study was to evaluate the influence of  $\beta$ -carotene on the level of oxidative DNA damage, misincorporation uracil and efficiency of DNA repair in lymphocytes of two groups of healthy individuals with antagonist diets.

## **2. Material and Methods**

### **2.1. Subject selection**

This study was approved by the ethical committee of the Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP, Brazil.

We studied the same 105 healthy adult volunteers (average age  $35,6 \pm 11,4$ , ranging from 19 and 66 years), 52 men and 53 women. All of the volunteer were nonsmokers, were not abusing alcohol, were not using prescription or recreational drugs and also were not using any vitamins and minerals supplementation. These individuals were divided in two groups: 1) 49 naturalistic individuals with a uncommon life style characterized by absence of stress, bucolic life and great consume of organic fruits, vegetables and juice and poor consume of industrialized foods (Group I); 2) 56 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 [31] 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. 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.

Only were selected individuals that have adopted the respective diet pattern for at least 10 years. Before the participation, the volunteers signed a declaration agreeing with the research.

## **2.2. Determination of Oxidative DNA Damage and level of uracil incorporate into DNA**

Data on oxidative DNA damage, uracil misincorporation into DNA, and DNA repair were described and accomplished by Prado et al in a parallel study [32]. Briefly, The alkaline Comet assay [33], modified with lesion-specific enzymes was used to detect single and double strand breaks, labile sites (SBs), oxidised purines and pyrimidines and uracil [34; 35]. All procedures were conducted in the dark to minimize spurious sources of DNA damage. Briefly, 10  $\mu$ l of the isolated lymphocytes suspension [36] ( $\cong 2 \times 10^4$  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<sup>0</sup>C. 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<sup>0</sup>C for 45 min with 100  $\mu$ L of endonuclease III (Endo

III - 1:1000; New England Biolabs Inc), 100  $\mu$ L of formamidopyrimidine-DNA glycosylase (FPG - 1:1000; New England Biolabs Inc), 100  $\mu$ L 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  $\cong$  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  $\mu$ L 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  $\beta$  - carotene on the DNA repair capability, 200  $\mu$ l of the freshly isolated lymphocytes were treated with 100  $\mu$ l of H<sub>2</sub>O<sub>2</sub> (100  $\mu$ M), for 30 minutes, in ice, and analysis of the level of damage was

evaluated. Then, after two wash in PBS to remove of H<sub>2</sub>O<sub>2</sub>, these aliquots were incubated at 37 °C for 30 minutes, and DNA damage was checked again.

### **2.3. Determination of level of $\beta$ - carotene**

Participants provided blood samples on the day of the baseline interview. Blood samples were centrifuged at 2600 rpm for 30 minutes and serum was separated and frozen at -80°C until analyzed for  $\beta$  - carotene. This micronutrient was measured using high-performance liquid chromatography (HPLC) as described by Yeum et al [37].

### **2.4. Statistical analysis**

The data obtained in the Comet Assay and HPLC were asymmetric distribution. Log transformations were applied to DNA damage distributions and serum  $\beta$ -carotene concentration to help meet normality distribution assumptions. The statistical analysis of the data obtained in HPLC consisted of applying the student's T test to compare the differences of serum  $\beta$ -carotene concentrations between the groups, and to establish the correlations between the serum  $\beta$ -carotene level and the various types of DNA damage, the Pearson correlation was applied. The index of significance adopted was 5%.



### 3. Results

The individuals of group I presented higher serum concentrations of  $\beta$ -carotene than the individuals of group II (Figure 1.).

Besides dietary intake, other factors as gender could be associated with carotenoid concentration. Therefore, we evaluated the possible influence of gender on the  $\beta$ -carotene serum concentration. We did not find difference of  $\beta$ -carotene serum concentration between genders in both groups (Figure 2.).

Table 1 shows the correlation between the serum concentrations of  $\beta$ -carotene and levels of SBs, purine and pyrimidine damaged, and misincorporation uracil on DNA, the levels of DNA damage induced with treatment of  $H_2O_2$  (100  $\mu$ M) and the DNA repair capability. In the total individuals there were a negative correlation between  $\beta$ -carotene and strand breaks (SBs) ( $r = -0,252$ ,  $p < 0,022$ ) and oxidised purines (SBs FPG) ( $r = -0,214$ ,  $p < 0,05$ ). In addition, with regard to the individuals of group II, there was a negative correlation between strand breaks (SBs) and  $\beta$ -carotene ( $r = -0,349$ ,  $p < 0,04$ ) and between misincorporated uracil (SBs UDG) and  $\beta$ -carotene ( $r = -0,369$ ,  $p < 0,029$ ). Individuals of group I did not present correlation between  $\beta$ -carotene on the levels of DNA damage.

We also evaluated, in the total individuals, if percentile of the level of serum  $\beta$ -carotene influenced the levels of DNA damage. No significant difference on levels of DNA damage were found between individuals that presented 25 percentile of serum concentration of  $\beta$ -carotene and 50 or between those

presented a percentile 50 and 75. However, the individuals that presented percentile 75 of serum concentration of  $\beta$ -carotene had higher levels of SBs ( $p=0.044$ ), SBs FPG ( $p=0.03$ ) and SBs Endo III ( $p=0.01$ ) than individuals with percentile 25.

The serum concentration of  $\beta$ -carotene did not influence the DNA repair capability in both groups.

#### **4. Discussion**

Several epidemiologic studies have shown an association between foods intake rich in carotenoids and low incidence of various cancers, such as lung, stomach, prostate, bladder, esophagus and larynx cancer [9]. Differences in fruit and vegetable intake can reflect in differences carotenoid levels measurement among populations, which could possibly explain part of the difference in cancer incidence [38]. However, reports in the literature involving  $\beta$ -carotene supplementation have been controversy.  $\beta$ -carotene supplementation have been associated both positively and negatively with cancer incidence, but in some studies no effect have been reported [29; 39; 40; 41; 42]. In the present study, our focus is on the  $\beta$ -carotene and on their influence on the oxidative DNA damage, misincorporated uracil and DNA repair capability in two groups of brazilian volunteers with antagonist diets.

In low concentrations of antioxidant, free radicals can damage cellular proteins and lipids and form DNA adducts contributing to carcinogenic [43]. In our

study, we found the negative correlation between serum concentration of  $\beta$ -carotene and SBs ( $r=-349$ ,  $p=0.04$ ) and uracil incorporation ( $r=-369$ ,  $p=0.029$ ) in individuals of the group II and between serum concentration of  $\beta$ -carotene and SBs ( $r=-252$ ,  $p=0.022$ ) and oxidised purines ( $r=-214$ ,  $p=0.05$ ) in the total individuals, meaning that these DNA damage increased with decreased of serum concentration of  $\beta$ -carotene. In accordance with our study, several studies reported an inverse correlation between certain nutrients/diets and carcinogenesis [27]. Our findings also are consistent with other published data, showing that  $\beta$ -carotene prevented DNA strand breaks in cells [28], since  $\beta$ -carotene can act as antioxidant, quenching singlet  $O_2$  and inhibiting other free radicals.

However, when  $\beta$ -carotene intake is high, it may act as a pro-oxidant, causing toxic effects, such as increased oxidative stress in the lungs of smokers [44]. We found a significant increase in SBs and purine and pyrimidines oxidized in the individuals that presented the 75th percentile of serum concentration of  $\beta$ -carotene, suggesting that high concentration of  $\beta$ -carotene could lead to a pro-oxidant effect. Observational epidemiological studies suggest that an ingestion of about 4 mg/day carotenoids, quantity present in a food rich in fruit and vegetables, may protect against cancer without leading to health risks [45]. These findings have generated recommendations about the ingestion of  $\beta$ -carotene (and other carotenoids), which should not exceed 10 mg/day, especially in the case of smokers [46].

Although a number of studies have reported protective effects of carotenoids against DNA damage [47; 48; 49; 50], we did not find influence of the  $\beta$ -carotene on the levels of DNA damage in individuals with great consumption of

organic fruits, vegetables and (group I), suggesting that other protective factors could be relationship to DNA damage level. Several studies have been reported the difficult to determine the real effect of nutrients isolated in chemoprevention cancer [51]. Since that the micronutrients could interact and lead to synergistic effect, as for example the interaction between the compounds poliphenolics and fiber diet that decreased the risk of colorectal cancers [45]. The combination of  $\beta$ -carotene with ascorbic acid and  $\alpha$ -tocopherol increases to protective effective, since such nutrients may interact in cellular environment and to potentiate the antioxidant defense [52] resulting, for example, in protection against oxidative damage in the DNA [22; 23; 24].

Several studies have been reported higher oxidative DNA damage level in male than in female, which was attributed to lower fruit intake for male [36; 53]. In our study, we did not found significantly differences of  $\beta$ -carotene serum concentration between genders, in both groups, suggesting a similar intake of fruit and vegetables. These findings could be relationship with findings reported by Prado et al [32] (in preparation, parallel study of our laboratory), that did not found statistically significant difference on DNA damage levels between genders, in both groups. Our findings agree with other published data, which also did not found differences on intake of fruit and vegetables or DNA damage level between genders [54; 55; 56].

In conclusion, our results provide evidence that the diet rich in fruit and vegetables, rich in  $\beta$ -carotene, can be capable of protecting cells against 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, the high concentration of  $\beta$ -carotene can lead to increase of DNA damage.

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## Legends

Figure1. Levels of  $\beta$ -carotene on group I (n= 49), and group II (n=56). Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The individuals of group I presented higher concentrations on serum of  $\beta$ -carotene than the individuals of group II \* p < 0.0001.

Figure2. Influence of gender on serum  $\beta$ -carotene concentration. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The carotenoid concentrations did not differed between the genders in both groups.

Table1. Correlations between  $\beta$ -carotene and levels of SBs, SBs FPG, SBs Endo III, SBs UDG, SBS H<sub>2</sub>O<sub>2</sub> e SBs H<sub>2</sub>O<sub>2</sub>R.

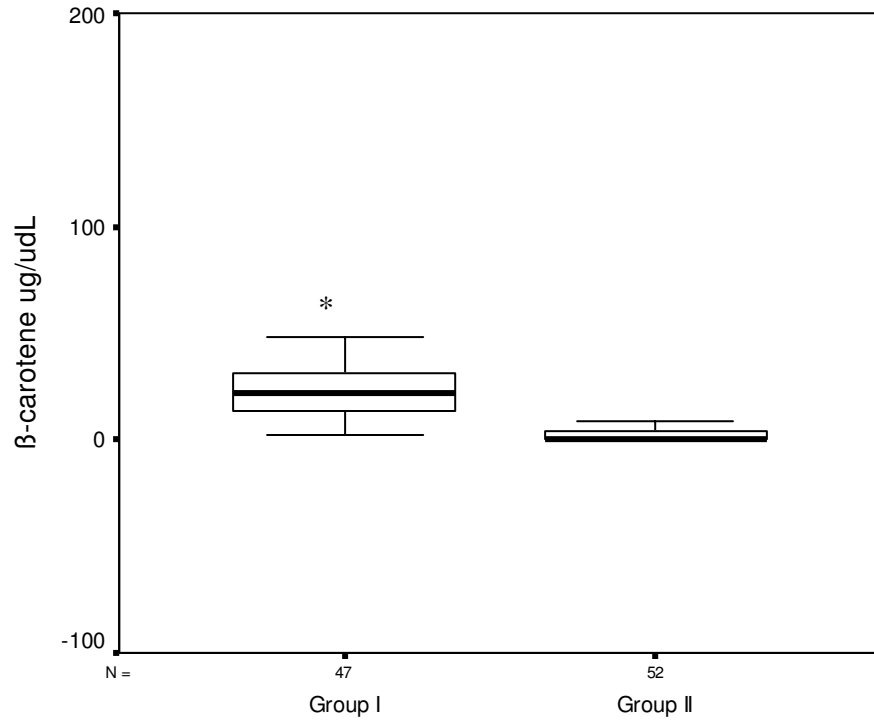


Figure1.

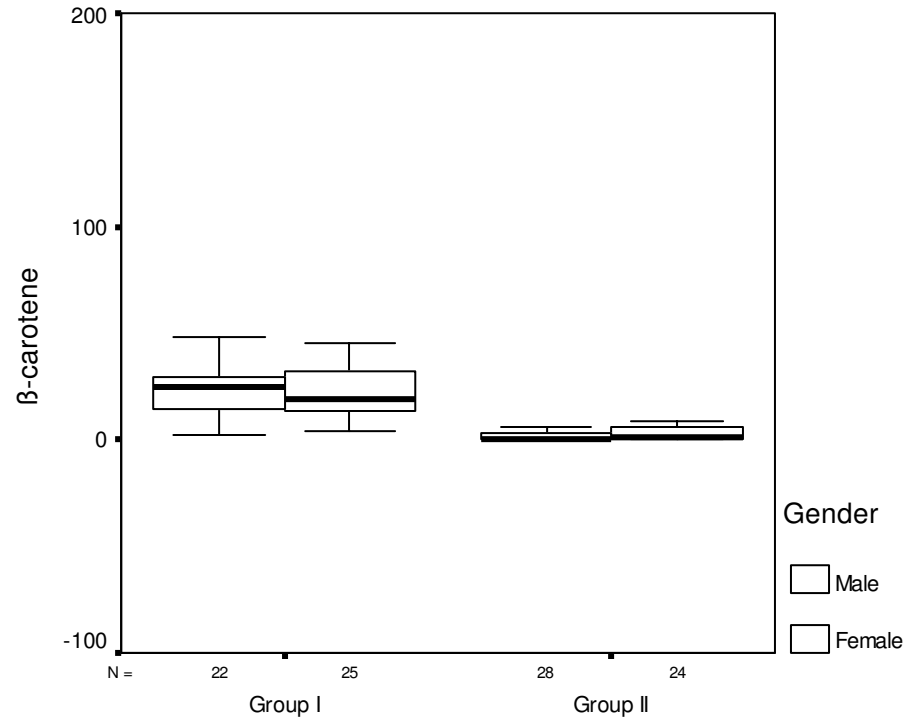


Figure2.

Table1.

Group I	Correlation	P
SBs	0,086	0,565
SBs FPG	0,071	0,636
SBs Endo III	0,085	0,570
SBs UDG	-0,050	0,739
SBs H2O2	0,038	0,802
SBs H2O2R	0,047	0,752
Group II		
SBs	-0,348*	0,04
SBs FPG	-0,122	0,483
SBs Endo III	-0,079	0,654
SBs UDG	-0,369*	0,029
SBs H2O2	-0,224	0,195
SBs H2O2R	-0,276	0,109
Total population		
SB	-0,252*	0,022
SBs FPG	-0,214*	0,05
SBs Endo III	-0,184	0,099
SBs UDG	-0,170	0,127
SBs H2O2	-0,023	0,836
SBs H2O2R	-0,048	0,668

**Manuscrito III**

# Influence of Lycopene on the Oxidative DNA Damage, Misincorporated Uracil and DNA Repair Capability

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*(Running Title):* Influence of Lycopene on DNA Damage and Repair

Key Words: Diet, oxidative DNA damage, uracil incorporation, DNA repair, comet assay, lycopene and carotenoids

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## Abstract

Lycopene is a natural pigment synthesized by plants and microorganisms, and it is one of the most potent antioxidants. Epidemiological studies showed the protective action of lycopene on cardiovascular diseases and various types of cancer. The present study evaluated the influence of lycopene on the level of oxidative DNA damage, misincorporated uracil and DNA repair capability in peripheral blood lymphocytes from two groups of individuals with antagonist diets: 1) 49 healthy individuals with a diet rich in organic products, whole grains, fruit and vegetables and poor in industrialized products (Group I); 2) 56 healthy individuals with diet rich in industrialized products and poor in fruit and vegetables (Group II). Oxidative DNA damage, uracil incorporation and DNA repair capability were assessed by the comet assay. The levels of lycopene in the serum were measured using high-performance liquid chromatography (HPLC). The individuals of group I presented higher serum concentrations of lycopene than the individuals of group II ( $p < 0.05$ ). The serum concentration of lycopene was correlated negatively with misincorporation uracil in both groups, with  $p = 0.044$  for group I and  $p = 0.010$  for group II. However, the individuals that presented percentile 75 of serum concentration of lycopene had higher levels of oxidised purines ( $p=0.03$ ) than individuals with percentile 25. The analysis of our results provide evidence that lycopene can protect against misincorporation of uracil on DNA. Nevertheless, the high concentration of lycopene can lead to increase of oxidative DNA damage.

## 1. Introduction

Diet certainly plays an important role in preventing cancer, but the mechanisms involved are still not clear [1]. The protective role of carotenoids against cancer has been credited, among other mechanisms, their antioxidant properties [2]. Several epidemiological studies have shown that individuals who consume large amounts of fruits and vegetables rich in carotenoids present a lower incidence of lung, stomach, breast, colon, liver and pharynx cancer [3].

Carotenoids are family of pigmented compound synthesized by plants and microorganisms and are responsible for the colors of the nature. However, humans need to obtain them from diet [4]. About 600 carotenoids already were isolated and characterized [5]. Besides, only about 40 are present in human diet, and about 12 carotenoids can be found in human blood and tissues in measurable concentrations [6]. The carotenoids are molecules compose for 40 carbons, which act as antioxidants combating free radicals that can cause damage to protein, lipids and DNA [7] and they have also been shown to protect low density lipoproteins (LDLs) against oxidation *in vitro* [8].

Among the carotenoids, lycopene is one of the most potent antioxidants [9]. This is a carotenoid acyclic, composed of atoms of carbon and hydrogen united by 11 dyads links together [10]. It is a natural pigment synthesized by plants and microorganism [11]. Although it is also found in some fruit as guava, watermelon and papaya, tomato is its main source [10]. Lycopene is an acyclic isomer of  $\beta$ -carotene, but without vitamin A activity [12]. Epidemiological and experimental studies showed the protective action of lycopene on cardiovascular diseases and

various types of cancer (mainly of the breast, prostate and colon) [10]. Although it is unknown exact mechanism by which the lycopene exercises such actions, there are the chances of its activity on the reductive singlet oxygen [9]; its protection against lesions caused by membrane radical nitrogen dioxide and hydrogen peroxide [13]; the increase of communications via gap intercellular junctions; inhibition of cellular proliferation interfering in reports cellular growth factors; stimulating the metabolism of xenobiotics and the modulation of the inflammatory process.

It is important to emphasize that the deficiencies of some nutrients may be reduced with interventions and relatively small investments in public health, and by strategies to improve the access, availability and consumption of foods rich in vitamins and minerals. The ingestion optimal/ideal of a given micronutrient may vary with age, genetic constitution, life style, cultural habit, among others. Measure the ideal level of ingestion of micronutrients and combat shortcomings, shall be the greatest challenge in the coming decades, since for each disease the ideal level of ingestion of the same micronutrient can vary significantly [14]. Since DNA damage suggests the early phase of carcinogenesis, it is interesting to know how the oxidative damage of DNA produced by free radicals is prevented by the antioxidant nutrients.

Thus, the reason for carrying out this study was to evaluate the influence of lycopene on the level of oxidative DNA damage, misincorporation uracil and efficiency of DNA repair in lymphocytes of two groups of healthy individuals with antagonist diets.

## **2. Material and Methods**

### **2.1. Subject selection**

This study was approved by the ethical committee of the Botucatu Medical School, São Paulo State University (UNESP), Botucatu, SP, Brazil.

We studied the same 105 healthy adult volunteers (average age  $35,6 \pm 11,4$ , ranging from 19 and 66 years), 52 men and 53 women, that was analyzed in preceding studied. All of the volunteer were nonsmokers, were not abusing alcohol, were not using prescription or recreational drugs and also were not using any vitamins and minerals supplementation. These individuals were divided in two groups: 1) 49 naturalistic individuals with a uncommon life style characterized by absence of stress, bucolic life and great consume of organic fruits, vegetables and juice and poor consume of industrialized foods (Group I); 2) 56 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 [15] 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. 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.

Only were selected individuals that have adopted the respective diet pattern for at least 10 years. Before the participation, the volunteers signed a declaration agreeing with the research.

## **2.2. Determination of Oxidative DNA Damage and level of uracil incorporate into DNA**

Data on oxidative DNA damage, uracil misincorporation into DNA, and DNA repair were described and accomplished by Prado et al [16] in a parallel study (in preparation). Briefly, The alkaline Comet assay [17], modified with lesion-specific enzymes was used to detect single and double strand breaks, labile sites (SBs), oxidised purines and pyrimidines and uracil [18; 19]. All procedures were conducted in the dark to minimize spurious sources of DNA damage. Briefly, 10  $\mu$ l of the isolated lymphocytes suspension [20] ( $\cong 2 \times 10^4$  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<sup>0</sup>C. 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<sup>0</sup>C for 45 min with 100  $\mu$ L of endonuclaease

III (Endo III - 1:1000; New England Biolabs Inc), 100  $\mu$ L of formamidopyrimidine-DNA glycosylase (FPG - 1:1000; New England Biolabs Inc), 100  $\mu$ L 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  $\cong$  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  $\mu$ L 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 lycopene on the DNA repair capability, 200  $\mu$ l of the freshly isolated lymphocytes were treated with 100  $\mu$ l of

H<sub>2</sub>O<sub>2</sub> (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<sub>2</sub>O<sub>2</sub>, these aliquots were

### **2.3. Determination of level of lycopene**

Participants provided blood samples on the day of the baseline interview. Blood samples were centrifuged at 2600 rpm for 30 minutes and serum was separated and frozen at -80 °C until analyzed for lycopene. This micronutrient was measured using high-performance liquid chromatography (HPLC) as described by Yeum et al [21].

### **2.4. Statistical analysis**

The data obtained in the Comet Assay and HPLC were asymmetric distribution. Log transformations were applied to DNA damage distributions and serum lycopene concentration to help meet normality distribution assumptions. The statistical analysis of the data obtained in HPLC consisted of applying the student's T test to compare the differences of serum lycopene concentrations between the groups, and to establish the correlations between the serum lycopene level and the various types of DNA damage, the Pearson correlation was applied. The index of significance adopted was 5%.

### 3. Results

Serum levels of lycopene were evaluated in both groups and are shown in Figure 1. The individuals of group I presented higher serum lycopene concentrations than the individuals of group II ( $p < 0.013$ ).

Besides dietary intake, other factors as gender could be associated with carotenoid concentration. Therefore, we evaluated the possible influence of gender on the lycopene serum concentration. We did not find difference of lycopene serum concentration between genders in both groups (Figure 2.).

Table 1. shows the correlation between the serum concentration of lycopene and levels of SBs, purine and pyrimidine damaged, misincorporation uracil on DNA, the levels of DNA damage induced with treatment of  $H_2O_2$  (100  $\mu M$ ) and the DNA repair capability. The serum concentration of lycopene was correlated negatively with misincorporation uracil in both groups with  $p = 0.044$  for group I and  $p = 0.010$  for group II.

We also evaluated, in the total individuals, if percentile of the level of serum lycopene influenced the levels of DNA damage. No significant difference on levels of DNA damage were found between individuals that presented 25 percentile of serum concentration of  $\beta$ -carotene and 50 percentile or between those presented a percentile 50 and 75. However, the individuals that presented percentile 75 of serum concentration of lycopene had higher levels of purine damaged ( $p = 0.03$ ) than individuals with percentile 25.



## 4. Discussion

The possible preventive role of lycopene in reducing the incidence of chronic diseases (mainly the cancer) has been examined in several epidemiological studies and reviews [12; 22], and lycopene is included among promising chemopreventive dietary substances [23]. In the present study, we investigated the influence of lycopene on the level of oxidative DNA damage, misincorporation uracil and efficiency of DNA repair in lymphocytes of two groups of healthy individuals with antagonist diets and in the total individuals.

Although epidemiologic studies continues to accumulate evidence that diets high in fruit and vegetables are associated with a reduced risk of chronic diseases, such as cardiovascular disease and cancer (24; 25; 26), our results showed that lycopene did not influence significantly on level of oxidative DNA damage and DNA repair capability in both groups, even the individuals of group I presenting a level higher serum lycopene when compared to individuals for group II. Our results are in agreement with the results reported by van den Berg *et al.* [27] and Møller *et al.* [28] that reported that individuals supplementation with 600g/day of fruit and vegetable intake did not altered levels of SBs, purine and pyrimidines oxidized, lipid peroxidation, oxidized/reduced glutathione ratio, and activity of glutathione *S*-transferases. This may indicate that more severe depletion of lycopene is required to elevate the levels of oxidative DNA damage and that the amount of lycopene ingested by individuals in group II may have been sufficient to maintain the DNA integrity. Another possible factor that can influenced our results could be that the bioavailability of antioxidants in fluid products (juices) and food products (puree) is

higher than in fresh fruit and vegetables [28], since the individuals in group II even ingested low amounts of fruit and vegetable, were mostly in the form of juices or offset by the great ingestion of derived primarily from tomato products, such as pizza, ketchup, spaghetti, and chili, relative rich in this carotenoids.

However, when carotenoids intake is high, it may act as a pro-oxidant, causing toxic effects, such as increased the state oxidative chronic in the lungs of smokers [29]. We found significant increase on purine oxidized (oxidative DNA damage) in the individuals that presented percentile 75 of serum concentration of lycopene, suggesting that high concentration of lycopene could lead to a pro-oxidant effect. Our results are in agreement with the results reported by Yeh et al. [30] showing that lycopene enhances UVA-induced oxidative stress in C3H cells, suggesting that under UVA irradiation, lycopene may produce oxidative products that are responsible for the pro-oxidant effects. This property may be a reason why lycopene contributed to induction of oxidative DNA damage in our study. In addition, the genotoxicity of chemopreventive agents can be modulated in combination with other DNA-damaging agents that are present in the environment [31]. Therefore, the chemopreventive activity of lycopene depends on the dose and environmental conditions.

Several studies have been reported that carotenoids, especially lycopene, may modulate the expression of various genes such as xenobiotic metabolism genes, DNA repair genes and apoptosis molecular pathway (32; 33; 34). In this study, the serum concentration of lycopene was correlated negatively with misincorporation uracil in both groups, suggesting a protective effect of lycopene.

We hypothesized that lycopene could induce this protective effect by modulation of gene expression from acid folic metabolism genes such as *TS* or *MTHFR*, which influenced the rate of conversion of dUMP to dTMP.

Our results indicate that lycopene concentrations were not affected by gender, although sex have been suggested as a factor associated to variation in the carotenoids concentrations in many studies. Several studies reported that the serum concentrations of lycopene were significantly higher in women than in men [35; 36; 37], since that women normally ingest more fruit and vegetable than men. But, in the present study, as both groups were well characterized, we see that the ingestion of fruits and vegetables or sources of lycopene were similar between men and women in both groups respectively, which can help explains our result.

In conclusion, our results provide evidence that lycopene can protect against misincorporation of uracil on DNA. Nevertheless, the high concentration of lycopene can lead to increase of DNA damage. Additional studies on the effect of fruit and vegetables on oxidative DNA damage in oxidative stress situations are needed to support a role for fruit and vegetables in the prevention of cancer. Nevertheless, the high concentration of lycopne can lead to increase of oxidative DNA damage.

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## Legends

Figure1. Levels of lycopene on group I (n= 49), and group II (n=56). Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The individuals of group I



presented higher concentrations on serum of lycopene than the individuals of group II \*  $p < 0.0001$ .

Figure2. Influence of gender on serum  $\beta$ -carotene concentration. Results are expressed in interquartile ranges within the box, with the median at the line, and the 5<sup>th</sup> and 95<sup>th</sup> percentiles at the whiskers. The carotenoid concentrations did not differed between the genders in both groups.

Table1. Correlations between lycopene and levels of SBs, SBs FPG, SBs Endo III, SBs UDG, SBS H2O2 e SBs H2O2R.

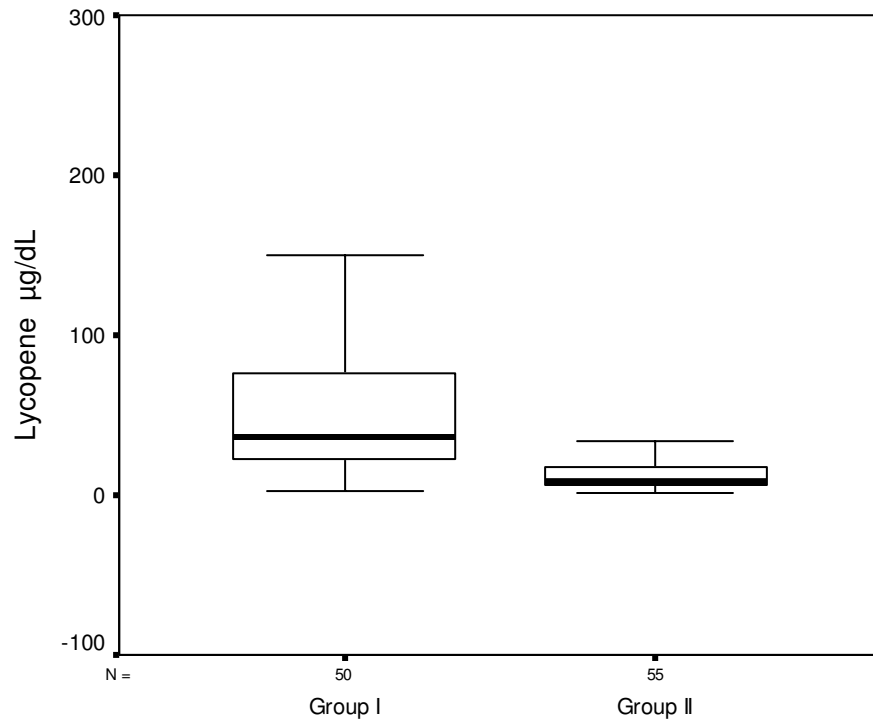


Figure1.

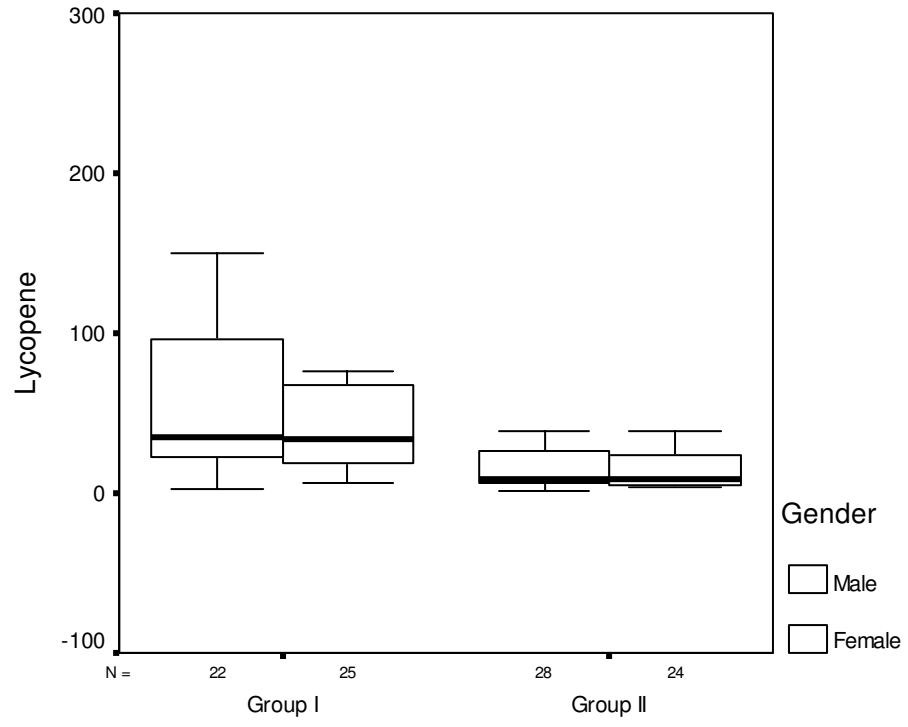


Figure2.

Table1.

Group I	Correlation (r)	P
SBs	-0.071	0.638
SBs FPG	0.051	0.733
SBs Endo III	0.061	0.686
SBs UDG	-0.285	0.044
SBs H2O2	0.021	0.888
SBs H2O2R	-0.002	0.989
Group II		
SBs	-0.047	0.738
SBs FPG	-0.116	0.411
SBs Endo III	-0.182	0.196
SBs UDG	-0.356	0.010
SBs H2O2	0.169	0.230
SBs H2O2R	0.159	0.259
Total population		
SBs	-0.131	0.197
SBs FPG	-0.203	0.056
SBs Endo III	-0.172	0.089
SBs UDG	-0.136	0.178
SBs H2O2	0.186	0.066
SBs H2O2R	0.113	0.267

#### IV. Conclusões finais

Com base nos resultados da influência de diferentes padrões alimentares sobre os níveis de danos oxidativos no DNA, os níveis de incorporação de uracila e a eficiência do sistema reparo do DNA e dos resultados da correlação entre o nível de  $\beta$ -caroteno e de licopeno plasmáticos e o nível de danos oxidativos no DNA de sangue periférico, pode-se concluir que:

- 1) Dieta rica em cereais integrais, frutas e legumes e pobres em produtos industrializados associados com um estilo de vida saudável pode proteger contra danos oxidativos no DNA;
- 2) Dieta rica em frutas e legumes, rico em  $\beta$ -caroteno, pode ser capaz de proteger as células contra danos no DNA, e conseqüentemente levar a um risco diminuído de desenvolvimento de câncer.
- 3) O Licopeno não influenciou danos oxidativos no DNA e a eficiência do sistema reparo do DNA em ambos os grupos, sendo necessários mais estudos para suportar o papel do licopeno na prevenção do câncer.
- 4) Em altas concentrações (percentil 75), tanto  $\beta$ -caroteno como licopeno podem levar ao aumento de danos no DNA, sugerindo que este efeito poderia aumentar o risco de desenvolvimento de câncer.

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## **Anexo 1**

### TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

#### EXPLICAÇÃO PARA O PACIENTE

A sociedade atual sofre uma alta taxa de doenças degenerativas crônicas, entre elas as doenças do coração, hipertensão, diabetes, doença de Alzheimer, doença de Parkinson e principalmente o câncer. Essa situação é reflexo da mudança dos hábitos alimentares, que passaram a ser mais ricos em produtos industrializados e contendo inúmeros aditivos químicos e pobres em grãos integrais, frutas e vegetais. Grande parte desses aditivos químicos alimentares podem causar quebras no DNA e o acúmulo dessas quebras leva a vários tipos de doenças, inclusive o câncer. Os alimentos integrais, as frutas e os vegetais contêm grande quantidade de fibras, vitaminas e nutrientes que são essenciais para a manutenção de uma boa saúde.

Em virtude disto o(a) Sr.(a) está sendo convidado a participar de um estudo que vai investigar se a sua alimentação pode danificar ou pode proteger o seu DNA. Caso o(a) Sr.(a) aceite participar do estudo, serão coletados 10 ml do seu sangue, através do braço, com seringa e agulhas descartáveis e estéreis, para verificarmos as quebras do DNA do seu sangue. Além disso, será preenchido um questionário, no qual o(a) senhor(a) nos dará várias informações sobre os seus hábitos alimentares.

Como o(a) senhor(a) deve acompanhar pela televisão, a genética e a medicina têm evoluído muito e por isso eu gostaria de pedir seu consentimento para usar o seu material genético em estudos futuros, que contribuirão, mais ainda, para o entendimento das doenças degenerativas crônicas e do câncer.

## TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

DEPARTAMENTO DE PATOLOGIA - FMB – BOTUCATU – SP

### I. Identificação do paciente ou responsável legal

Nome:		
RG:	Sexo: <input type="checkbox"/> M <input type="checkbox"/> 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 DE MICRONÚCLEOS EM LINFÓCITOS”
2. Pesquisadores-Responsáveis: Dr. Marcelo Sady Plácido Ladeira e Dra. Daisy Maria Fávero Salvadori, Departamento de Patologia – Faculdade de Medicina de Botucatu, UNESP, Botucatu - SP.
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: 10 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, 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 à 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 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: Dra. Daisy Maria Fávero Salvadori

Endereço: Departamento de Patologia – Faculdade de Medicina de Botucatu, UNESP, Rubião Júnior S/N, Botucatu – SP.

Telefone: (14) 38116376

Nome: Dr. Marcelo Sady Plácido Ladeira.

Endereço: Departamento de Clínica médica – Faculdade de Medicina de Botucatu, UNESP, Rubião Júnior S/N, Botucatu – SP.

Telefone: (14) 38116376

## 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

## Inquérito de frequência alimentar semi-quantitativo

### *Dados pessoais*

Nome:

Idade:

Sexo:

Peso:

Altura:

Pratica atividade física:

Observações e história clínica:

- Inquérito proposto com os seguintes objetivos: (i) conhecer perfil dos hábitos alimentares; (ii) conhecer a frequência e estimar a quantidade consumida de alimentos fonte de vitaminas interferentes no processo oxidativo/ prevenção do dano oxidativo; (iii) conhecer a frequência e estimar a quantidade de alimentos fonte de carotenóides, retinol e vitamina E.









**Anexo 3****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 redução 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	Frequência

4. As questões seguintes relacionam-se ao seu hábito alimentar usual no período de um ano. Para cada quadro abaixo responda, por favor, a frequência 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 círculo da primeira coluna (N= nunca come). Não deixe itens em branco.

Grupo de alimentos	Com que frequência você costuma comer?			Qual o tamanho de sua porção em relação à porção média?												
	Quantas vezes você come:			Unidade												
	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 (M)												
				Porção média de referência												
				Sua porção												
				P= menor que a porção média M= igual à porção média G= maior que a porção média E= bem maior que a porção média												
<b>Sopas e massas</b>	Quantas vezes você come			Unidade			Porção média (M)	Sua porção								
Sopas (de legumes, canja, creme, etc)	N	1	2	3	4	5	6	7	8	D	S	M	1 concha média (150g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salgados fritos (pastel, coxinha, risólis, bolinho)	N	1	2	3	4	5	6	7	8	D	S	M	1 unidade grande (80g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salgados assados (esfiha, bauruzinho, torta)	N	1	2	3	4	5	6	7	8	D	S	M	2 unidades ou 2 pedaços médios (140g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Macarrão com molho sem carne	N	1	2	3	4	5	6	7	8	D	S	M	1 prato raso (200g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Macarrão com molho com carne, lasanha, nhoque	N	1	2	3	4	5	6	7	8	D	S	M	1 escumadeira ou 1 pedaço pequeno (110g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Pizza, panqueca	N	1	2	3	4	5	6	7	8	D	S	M	2 fatias pequenas ou 2 unidades (180g)	P	M	G
	9	10									A				E	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>								<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

	<input type="radio"/> <input type="radio"/>	<input type="radio"/>		<input type="radio"/>
Polenta cozida ou frita	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 colheres de sopa ou 2 fatias pequenas (70g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Cereais integrais</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Aveia, quinoa, linhaça, trigo	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 colheres de sopa (40g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
arroz	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 escumadeiras médias (120g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Pães e biscoitos</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Pão francês, pão de forma, pão doce, torrada	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 unidade ou 2 fatias (50g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Pão de forma integral	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 fatias (50g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Biscoito sem recheio	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	4 unidades (24g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Biscoito recheado, waffer, amanteigado	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 unidades (41g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Pães e biscoitos</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Bolo simples	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 fatia média (60g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Bolo recheado	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 fatia média (60g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Hortaliças/verduras e frutas verdes</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Alface	N 1 2 3 4 5 6 7 8 9 10	D S M	3 folhas médias	P M G

	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	(30g)	<input type="radio"/> E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Almeirão	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 folhas médias (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Agrião	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 prato de sobremesa (38g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Brócolis	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 ramo ou 2 colheres de sopa (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Chicória crua	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 prato de sobremesa (38g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Chicória cozida	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de servir (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Couve manteiga crua	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 prato de sobremesa (38g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Couve manteiga cozida	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de servir (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Chuchu	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de sopa cheia (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Mostarda	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 folhas médias (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Rúcula	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 prato de sobremesa (38g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Espinafre	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de servir (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>

																			<input type="radio"/>	
Pepino	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 colher de sopa cheia (30g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Pimentão	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 colher de sopa cheia (30g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Abobrinha	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 colher de sopa cheia (30g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Quiabo	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 colher de sopa cheia (30g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Abacate	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	2 colheres de sopa cheia (90g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Azeitona	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	6 unidades (25g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Figo	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 unidade média (50g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Kiwi	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	1 unidade média (40g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
<b>Verduras/ Hortaliças e frutas verdes</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção						
Uva	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	10 gomos (80g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
Jiló	N 9	1 10	2	3	4	5	6	7	8	D  <input type="radio"/>	S A  <input type="radio"/>	M  <input type="radio"/>	2 colheres de sopa (32g)	P  <input type="radio"/>	M E  <input type="radio"/>	G  <input type="radio"/>				
<b>Verduras/Hortaliças amarelo-</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção						



	<input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	<input type="radio"/> A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	média (150g)	<input type="radio"/> E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Verduras/ Hortaliças e frutas vermelhas</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Tomate	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 fatias médias (40g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Molho de tomate caseiro/lata/caixa	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1,5 colher de sopa (25g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Extrato de tomate	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1,5 colher de sopa (25g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Vinagrete	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	3 colheres de sopa (45g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Rabanete	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de sopa cheia (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Acerola	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	10 unidades (50g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Caqui	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 unidade média (120g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Goiaba	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 unidade grande (225g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Pitanga	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	10 unidades (50g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Melancia	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 fatia média (150g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>



Morango	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	7 unidades médias (84g)	P M G E ○ ○ ○ ○
<b>Hortaliças/verduras vermelho- arroxeadas</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Beterraba	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	1 colher de sopa cheia (30g)	P M G E ○ ○ ○ ○
Repolho	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	2 colheres de sopa (30g)	P M G E ○ ○ ○ ○
Berinjela	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	1 colher de sopa cheia (30g)	P M G E ○ ○ ○ ○
Uva	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	10 gomos (70g)	P M G E ○ ○ ○ ○

**Verduras/  
Hortaliças e frutas  
branco-  
amareladas**

	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Couve-flor	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	1 ramo (30g)	P M G E ○ ○ ○ ○
Acelga crua	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	1 prato de sobremesa (38g)	P M G E ○ ○ ○ ○
Acelga cozida	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	1 colher de servir (30g)	P M G E ○ ○ ○ ○
Repolho	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M A ○ ○ ○ ○	2 colheres de sopa (30g)	P M G E ○ ○ ○ ○
Melão	N 1 2 3 4 5 6 7 8 9 10	D S M A	1 fatia média (150g)	P M G E

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Maçã	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade média (110g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
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Banana nanica	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade média (100g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
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Banana prata	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade média (86g)	P M G E			
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Banana maçã	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade média (75g)	P M G E			
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Goiaba	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade grande (225g)	P M G E			
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Pêra	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 unidade média (110g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
Jabuticaba	N 1 2 3 4 5 6 7 8 9 10								D S M A			10 unidades (70g)	P M G E			
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	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
<b>Tubérculos</b>	Quantas vezes você come								Unidade			Porção média (M)		Sua porção		
Mandioca cozida	N 1 2 3 4 5 6 7 8 9 10								D S M A			1 escumadeira cheia (90g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
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Mandioca frita	N 1 2 3 4 5 6 7 8 9 10								D S M A			2 colheres de servir cheias (100g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
Batata frita	N 1 2 3 4 5 6 7 8 9 10								D S M A			2 colheres de servir cheias (100g)	P M G E			
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	

Batata cozida	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	escumadeira cheia (90g)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Batata doce frita	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 colheres de servir cheias (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Batata doce cozida	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	escumadeira cheia (90g)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Tubérculos</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Farinha de mandioca, farofa, cuscuz, tapioca	N 9	1 10	2	3	4	5	6	7	8	D	S	M	3 colheres de sopa (40g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Bebidas</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Suco de tomate	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de laranja	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de limão	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de goiaba	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de melancia	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de melão	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	(80ml)	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de acerola	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de maracujá	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Suco natural de abacaxi	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Suco natural de uva	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Suco natural de Pêssego	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Suco natural de Cajú	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Suco natural de morango	N 9	1 10	2	3	4	5	6	7	8	D	S	M	½ copo americano (80ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
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Cerveja	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 latas (700ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Vinho	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 taça pequena (50ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Café ( ) c/ ( ) s/ açúcar	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 xícaras de café (90ml)	P	M	G
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>						<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Chá ( ) c/	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 xícaras de	P	M	G

açúcar ( ) s/ açúcar	9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○		A		café (90ml)	E		
Refrigerantes	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1 copo de requeijão (240ml)	P M G	E	
Suco industrializado	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1 copo de requeijão (240ml)	P M G	E	
<b>Leite e derivados</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção				
Leite ( ) integral ( ) desnatado ( ) Semi-desn.	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		½ copo de requeijão (125ml)	P M G	E	
<b>Leite e derivados</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção				
Leite fermentado	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1 pote pequeno (90ml)	P M G	E	
iogurte ( ) integral ( ) desnat. ( ) semi-d.	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1 unidade pequena (140g)	P M G	E	
Queijo amarelos	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1,5 fatias grossas (30g)	P M G	E	
Queijos brancos (ricota, minas)	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		1 fatia média (30g)	P M G	E	
Requeijão	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		3 pontas de faca (15g)	P M G	E	
Manteiga ( ) comum ( ) light	N 1 2 3 4 5 6 7 8 9 10 ○ ○ ○ ○ ○ ○ ○ ○ ○ ○ ○	D S M	A		3 pontas de faca (15g)	P M G	E	

Margarina( )comum ( )light	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	3 pontas de faca (15g)	P M G E O O O O
<b>Leguminosas e ovos</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Amendoim	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	2 punhados (60g)	P M G E O O O O
Feijão	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	1 concha média (86g)	P M G E O O O O
Lentilha	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	1 colher de servir (35g)	P M G E O O O O
Grão-de-bico	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	1 colher de servir (35g)	P M G E O O O O
Guandu	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	1 colher de servir (35g)	P M G E O O O O
Vagem	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	1 colher de sopa cheia (30g)	P M G E O O O O
Soja	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	1 colher de servir (35g)	P M G E O O O O
Ervilha	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	1 colher de servir (35g)	P M G E O O O O
Ovo (cozido, frito)	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	1 unidade (50g)	P M G E O O O O
<b>Carnes</b>	Quantas vezes você come	Unidade	Porção média	Sua porção

Carne bovina	N 9	1 10	2	3	4	5	6	7	8	D	S	M	(M) 1 bife média ou 2 pedaços (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Hambúrguer, nuggets, almôdega	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 unidade média (60g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Frango( ) com pele	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 pedaço ou 1 filé pequeno (60g)	P	M	G
( ) sem pele	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carne suína	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 fatia média (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Vísceras</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Fígado, coração, língua, estômago, etc.	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 bife médio ou 2 pedaços (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Pescados, frutos do mar</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Bacalhau, caçã, salmão	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 filé pequeno ou 1 posta pequena (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lambari, merluza, pintado	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 filé pequeno ou 1 posta pequena (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Sardinha, atum	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 filé pequeno ou 1 posta pequena (100g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Embutidos, processados, defumados</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Presunto	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 fatias médias (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Salame	N 9	1 10	2	3	4	5	6	7	8	D	S	M	4 fatias (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mortadela	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 fatias médias (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Lingüiça	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 gomo médio (60g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Peito de peru	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 fatias médias (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salsicha	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 fatias médias (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Bacon	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 pedaços pequenos (40g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Carne seca, carne de sol	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 pedaços pequenos (40g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Oleaginosas</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Amêndoa	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 colheres de sopa (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Avelã	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 colheres de sopa (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Castanha	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 colheres de sopa (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>



Castanha-do-pará	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 colheres de sopa (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Noz	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 colheres de sopa (30g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Óleos</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Soja, girassol, canola, milho	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 fio (4ml)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Azeite	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 fio (4ml)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Temperos naturais</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Salsa, cebolinha, tomilho, louro, orégano, alecrim, coentro, manjericão	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 colher de sopa (3,8g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
<b>Doces e sobremesas</b>	Quantas vezes você come	Unidade	Porção média (M)	Sua porção
Açúcar, mel, geléia	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	½ colher de sopa (6g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Chocolate, bombom, brigadeiro	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 barra pequena (25g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Achocolatado em pó (adicionado ao leite)	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	2 colheres de sopa (25g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>
Sobremesas, doces, tortas e pudins	N 1 2 3 4 5 6 7 8 9 10 <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>	D S M A <input type="radio"/> <input type="radio"/> <input type="radio"/>	1 pedaço ou 1 fatia média (60g)	P M G E <input type="radio"/> <input type="radio"/> <input type="radio"/> <input type="radio"/>

Sorvetes cremosos	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 bola grande (70g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Doce de frutas (calda/barra)	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 colher grande ou 2 unidades (70g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
<b>Outros</b>	Quantas vezes você come									Unidade			Porção média (M)	Sua porção		
Glutamato monossódico (ex: sazon, ajinomoto)	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 pitada (0,35g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Adoçante	N 9	1 10	2	3	4	5	6	7	8	D	S	M	3 gotas	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Balas ou chicletes	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 unidade	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
				<input type="radio"/>	<input type="radio"/>	<input type="radio"/>				<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Enlatados	N 9	1 10	2	3	4	5	6	7	8	D	S	M	2 colheres de sopa (60g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Salgadinhos industrializados	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 pacote (30g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Maionese	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 colher de chá (4g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Mostarda	N 9	1 10	2	3	4	5	6	7	8	D	S	M	1 colher de chá (4g)	P	M	G
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>							<input type="radio"/>	<input type="radio"/>	<input type="radio"/>		<input type="radio"/>	<input type="radio"/>	<input type="radio"/>