**MARINA APOCALYPSE NOGUEIRA PEREIRA**

# EFFECT OF THE SUPPLEMENTATION WITH DIFFERENT SELENIUM DIETARY SOURCES ON THE EVOLUTION OF MAMMARY TUMOR INDUCED BY 4T1 CELLS INOCULATION IN MICE

**LAVRAS - MG**
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Dissertação apresentada à Universidade Federal de Lavras, como parte das exigências do Programa de Pós-Graduação em Ciências Veterinárias, área de concentração em Fisiologia e Metabolismo Animal, para a obtenção do título de Mestre.

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**MARINA APOCALYPSE NOGUEIRA PEREIRA**

# EFEITO DA SUPLEMENTAÇÃO DE DIFERENTES FONTES DIETÉTICAS DE SELÊNIO NA PROGRESSÃO DE TUMOR MAMÁRIO INDUZIDO POR INOCULAÇÃO DE CÉLULAS 4T1 EM CAMUNDONGOS

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APROVADA em 05 de novembro de 2018.

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Coorientador

**LAVRAS - MG**
**2019**

*A todos aqueles que se sentem encantados por conhecimento.*

*Dedico*

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*“Nenhum de nós é tão bom quanto todos nós juntos.”*

*(Autor desconhecido)*

## RESUMO

O câncer de mama é o mais prevalente entre as mulheres no Brasil e no mundo. Apesar dos avanços em métodos de diagnóstico precoce e tratamento, a morbidade e mortalidade deste tipo de câncer ainda são altas. Alguns micronutrientes tem sido estudados quanto ao seu potencial de inibição ou retardo da progressão de diversas neoplasias malignas. A associação entre os minerais (micronutrientes essenciais ao funcionamento celular) e o câncer de mama ainda foi pouco estudada e, dentre estes minerais, destaca-se o selênio (Se). Objetivou-se avaliar a influência da suplementação de diferentes fontes de Se na progressão de carcinoma mamário experimental induzido por inoculação de células 4T1 em camundongos fêmeas da linhagem *Balb-c*. Um total de 30 animais foi selecionado e cada camundongo recebeu uma injeção subcutânea no flanco para inoculação das células tumorais ($1 \times 10^6$ células 4T1 diluídas em 0.1 mL de solução tampão fosfato). O crescimento tumoral foi avaliado a cada 24 horas e ao quinto dia todos apresentavam tumores palpáveis. A partir desse momento, os animais foram aleatoriamente distribuídos em três grupos experimentais que receberam respectivamente: dieta com teor recomendado de Se (Se-adequado) 0.15 ppm; dieta enriquecida com selenometionina (SeMet) 1.4 ppm; ou dieta enriquecida com castanha do Brasil (Se-Castanha) 1.4 ppm por 28 dias. Nesse período avaliou-se o crescimento tumoral a cada 48 horas. No 29º dia os animais foram eutanasiados para análise de concentração sanguínea de Se bem como para quantificação da atividade enzimática de glutationa peroxidase (GPx) hepática. Nos primeiros 8 dias após início do tratamento dietético, o crescimento tumoral foi significativamente menor ($p < 0.05$) nos grupos suplementados com Se (SeMet e Se-Castanha) quando comparados à dieta Se-adequado. Contudo, esta proteção não foi mantida nas semanas seguintes e não houve efeito das dietas sobre o crescimento total do tumor em 28 dias. O grupo SeMet apresentou concentração média de Se no sangue de 0.507 µg/mL, significativamente maior ($p < 0.05$) do que o grupo Se-adequado (0.342 µg/mL), porém não diferindo do grupo Se-Castanha (0.461 µg/mL), que apresentou valor intermediário. As atividades médias de GPx nos grupos Se-adequado, SeMet e Se-Castanha foram, respectivamente, 6663.583, 7486.480 e 7862.332 nmo/min/mL, não havendo diferença significativa entre estes. Não houve correlação entre a concentração de Se no sangue, a atividade da GPx hepática e o crescimento tumoral. Concluiu-se que a suplementação dietética com Se (seja através de SeMet ou através de castanha do Brasil) reduziu o crescimento do tumor nos estágios iniciais. Estudos adicionais são encorajados visando esclarecer os mecanismos desencadeados pela ingestão de Se nos diferentes estágios da carcinogênese.



**Palavras-chave:** Neoplasias. Antioxidantes. Crescimento tumoral. Selenoproteína P. Glutationa peroxidase. Nutrição. Micronutrientes. Minerais. *Bertholletia.*

## ABSTRACT

Breast cancer is the most prevalent type in women from Brazil and worldwide. Despite the advances in early diagnosis and treatment methods, this kind of cancer still presents high morbidity and mortality. Some micronutrients have been studied because of their potential effect as inhibitors of malignant neoplasms evolution. The association between minerals (micronutrients that are essential for cellular functions) and breast cancer has not been much studied yet and, among these minerals, selenium (Se) stands out. This study aimed to evaluate the effect of the supplementation with different Se sources on the evolution of experimental mammary carcinoma induced by 4T1 cells inoculation in *Balb-c* female mice. Thirty animals were selected and each mice received a subcutaneous injection on the flank for tumor cells inoculation ($1 \times 10^6$ 4T1 cells diluted in 0.1 mL of phosphate buffered saline). Tumor growth was measured every 24 hours and at the fifth day all animals had palpable tumors. From then on, animals were randomly distributed into three experimental groups receiving respectively: diet containing recommended Se levels (Se-adequate) 0.15 ppm; diet enriched with selenomethionine (SeMet) 1.4 ppm; or diet enriched with Brazilian nuts (Se-Nuts) 1.4 ppm for 28 days. During that period, tumor growth was evaluated every 48 hours. At the $29^{th}$ day animals were euthanized for Se blood concentration analysis and for quantification of hepatic glutathione peroxidase (GPx) enzymatic activity. At the first 8 days after initiation of dietary treatment, tumor growth was significantly lower ($p < 0.05$) in Se-supplemented groups (SeMet and Se-Nuts) when compared to Se-adequate diet. However, the protection was not maintained at the following weeks and there was no effect of diets on tumor total growth through 28 days. SeMet group presented average Se blood concentration of 0.507 µg/mL, significantly higher ($p < 0.05$) than the Se-adequate group (0.342 µg/mL), but with no difference from Se-Nuts group (0.461 µg/mL), which presented an intermediate value. Average hepatic GPx enzyme activities in the Se-adequate, SeMet and Se-Nuts groups were, respectively, 6663.583, 7486.480 e 7862.332 nmo/min/mL, and no significant difference was observed between them. There was no correlation between Se blood concentration, GPx activity and tumor growth. It was concluded that dietary Se supplementation (by both SeMet or Brazilian nuts) reduced tumor growth during the initial stages. Additional studies are encouraged in order to elucidate mechanisms promoted by Se intake in the different carcinogenic stages.



**Keywords:** Neoplasms. Antioxidants. Tumor growth. Selenoprotein P. Glutathione peroxidase. Nutritional sciences. Micronutrients. Minerals. *Bertholletia*.

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**SEGUNDA PARTE – ARTIGO 1**

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# PRIMEIRA PARTE

## 1 INTRODUÇÃO

No Brasil, o câncer representa um importante problema de saúde pública devido a suas altas incidência e taxa de mortalidade (INCA, 2017). Além dos fatores genéticos (BRODERICK et al., 2007; JAEGER et al., 2008; WANG et al., 2008), bem como a exposição a fatores tóxicos tais como o cigarro e o álcool (BROWNSON et al., 1992; RITCHIE et al., 2001; PELUCCHI et al., 2006), a evolução do câncer está muito relacionada com a sobrecarga e o estresse oxidativo (SOTGIA; MARTINEZ-OUTSCHOORN; LISANTI, 2011), levando a danos no DNA que, se não reparados, podem resultar em mutações em genes supressores de tumor (KANG, 2002). Mas o estresse oxidativo pode ser modulado por alimentos (ZAIDI; BANU, 2004; STOCKLER-PINTO et al., 2010; STOCKLER-PINTO et al., 2014) e, dos vários nutrientes que exibem potenciais ações antioxidante e anticancerígena, destaca-se o selênio (Se). Sabe-se que em diversas regiões do mundo a baixa ingestão dietética desse elemento tem sido associada ao aumento na incidência de diferentes tipos de câncer (SHAMBERGER; FROST, 1969; SCHRAUZER; RHEAD, 1971; WILLETT et al., 1983). Assim, a avaliação dos efeitos de sua ingestão através de suplementos ou de alimentos com elevado teor desse mineral podem abrir novas perspectivas para prevenção de neoplasias.

As principais fontes de Se na dieta provêm tanto de vegetais quanto de carne, leite e ovos (RAYMAN, 2008; MEHDI et al., 2013; DUNTAS; BENVENGA, 2014; ROMAN; JITARU; BARBANTE, 2014). Contudo, é importante ressaltar que nem sempre a quantidade ingerida do nutriente prediz, por si só, a atuação do elemento nas funções vitais do organismo (SANDSTROM, 2001; HUNT, 2003; BROWNIE, 2006; HOLST; WILLIAMSON, 2008). A biodisponibilidade do Se é variável de acordo com as diferentes fontes, o status de Se do indivíduo, sua condição fisiológica, a espécie animal, os demais componentes da dieta, dentre outros fatores (ULLREY, 1987; BATES et al., 2002; SURAI, 2006; COMINETTI et al., 2011).

O fígado é o principal órgão regulador do Se (BURK; HILL, 2015), sendo, portanto, algumas vezes utilizado em experimentos para indicar o status de Se corporal através da quantificação de Se e/ou da expressão ou atividade da glutationa peroxidase tipo 1 (GPx-1) (SURAI, 2006; CHEN et al., 2013). Este órgão sintetiza a proteína transportadora de Se, chamada selenoproteína P (SelP), permitindo a distribuição de Se para os demais tecidos, de

modo que estes sintetizem suas respectivas selenoproteínas de acordo com suas necessidades (BURK; HILL, 2005; BURK; HILL, 2015).

Sabe-se que muitas funções biológicas do Se estão associadas a diferentes selenoproteínas (BRIGELIUS-FLOHE et al., 1994; BRIGELIUS-FLOHE, 1999; RAYMAN, 2000; PAPP et al., 2007; RAYMAN, 2009; STEINBRENNER; SIES, 2009; KASAIKINA; HATFIELD; GLADYSHEV, 2012; KIPP et al., 2012; RAYMAN, 2012; BRIGELIUS-FLOHE; MAIORINO, 2013; KUMAR; PRIYADARSINI, 2014; LABUNSKYY; HATFIELD; GLADYSHEV, 2014; TOUAT-HAMICI et al., 2014; TSUJI et al., 2015; DKHIL et al., 2016; STEINBRENNER; SPECKMANN; KLOTZ, 2016; WROBEL; POWER; TOBOREK, 2016; HOSNEDLOVA et al., 2017) e estudos mostram que os efeitos anticâncer do Se envolvem múltiplos mecanismos, incluindo ação antioxidante, atividade imunoestimulatória, inibição da proliferação de células tumorais, e modulação da via Wnt, do fator de crescimento endotelial vascular (VEGF) plasmático e da proteína supressora de tumor p53 (DONG et al., 2002; SMITH et al., 2004; RAYMAN, 2005; BRIGELIUS-FLOHE; KIPP, 2009; KIPP et al., 2009; BARRETT et al., 2012; FERGUSON et al., 2012; RESZKA, 2012; SANMARTIN et al., 2012; GUO; HSIA; CHEN, 2013; MEHDI et al., 2013; WALLENBERG; MISRA; BJORNSTEDT, 2014; FAGHFURI et al., 2015; FERNANDES; GANDIN, 2015; MISRA et al., 2015; TOBE et al., 2015). Contudo, poucos experimentos elucidaram os mecanismos antitumorais do Se oriundo da castanha do Brasil (*Bertholletia excelsa*), fonte vegetal relatada como sendo potencialmente rica em Se (DUMONT; VANHAECKE; CORNELIS, 2006; THOMSON et al., 2008; YANG, 2009; COMINETTI et al., 2012; CARDOSO et al 2017).

Recentemente reportou-se que selenometionina (SeMet), uma forma orgânica de Se, ingerida por camundongos em dose suplementar por três meses antes de inoculação tumoral 4T1 e mantida pelos 30 dias posteriores à indução, foi capaz de reduzir o crescimento do tumor mamário primário quando comparada ao crescimento do grupo deficiente em Se. Já o grupo que ingeriu Se na forma inorgânica, administrado em dose suplementar pelo mesmo período, não teve menor crescimento do tumor primário e ainda apresentou aumento da carga metastática total (CHEN et al., 2013).

O câncer de mama é o tipo que mais acomete mulheres no Brasil e no mundo (TORRE et al., 2016) e, dentre as taxas de mortalidade em pacientes oncológicos, o câncer mamário representa cerca de 6% das mortes (BRAY et al., 2018). Assim, no presente projeto, objetivou-se avaliar a ação da suplementação de diferentes fontes de Se na progressão de carcinoma mamário 4T1, por meio de: (1) comparação do crescimento tumoral, (2)

comparação do nível de Se em sangue total, e (3) avaliação da atividade enzimática de GPx-1 hepática.

# 2 REFERENCIAL TEÓRICO

## 2.1 Câncer

Define-se câncer como um grupo de doenças em que células anormais apresentam alterações nas taxas de mitose e na expressão de algumas proteínas, o que leva a mudanças em sua morfologia. Assim, em estágios mais avançados, essas células passam a invadir tecidos adjacentes, podendo alcançar o sangue ou a corrente linfática, o que permite futura colonização de outros órgãos e caracteriza as chamadas metástases (SATO et al., 2016).

Em 2018, foram estimadas 9,6 milhões de mortes por câncer, caracterizando essa patologia como a segunda maior causa de mortes no mundo (OMS, 2018). As neoplasias em geral resultam de um processo multifatorial (RITCHIE et al., 2001) que envolve condição nutricional (DOLL; PETO, 1981; DOLL, 1992; DONALDSON, 2004), genética (BRODERICK et al., 2007; JAEGER et al., 2008; WANG et al., 2008), exposição a substâncias (BROWNSON; CHANG; DAVIS, 1992; PELUCCHI et al., 2006) e/ou inflamações crônicas (OHSHIMA; BARTSCH, 1994; COUSSENS; WERB), dentre outros fatores.

Estudos sugerem que as espécies reativas de oxigênio (ROS) também possuem um papel no desenvolvimento de câncer (BENHAR; ENGELBERG; LEVITZKI, 2002; SANDER et al., 2004; BRANDON; BALDI; WALLACE, 2006), e o desbalanço entre sua produção e a capacidade antioxidante do organismo caracteriza o chamado estresse oxidativo (AMBROSONE et al., 1999). Esse estresse causa danos no DNA que, se não reparados, ou se não houver apoptose celular, podem levar a mutações em diferentes genes como, por exemplo, genes supressores de tumor (KANG, 2002). Assim a sobrecarga oxidativa tem sido relacionada a um maior risco (BAI et al., 2007; SHAHAR et al., 2008) bem como a uma maior progressão neoplásica (GOH et al., 2011; SOTGIA; MARTINEZ-OUTSCHOORN; LISANTI, 2011).

### 2.1.1 Câncer de mama

Dentre os diferentes tipos de câncer existentes, sabe-se que o mamário é o tipo que mais acomete as mulheres tanto no Brasil quanto globalmente (TORRE et al., 2016; BRAY et al., 2018). Além disso, dentre as taxas de mortalidade em pacientes oncológicos, o câncer de mama representa cerca de 6% das mortes (FERLAY et al., 2015; BRAY et al., 2018).

Os prognósticos mais desfavoráveis estão muito relacionados às ocorrências de metástases. Apesar dos avanços na compreensão das bases moleculares e genéticas do câncer, as metástases permanecem sendo a causa de mais de 90% da mortalidade relacionada ao câncer (WEIGELT; PETERSE; VAN'T VEER, 2005). Metástases tumorais resultam de um processo complexo que envolve migração celular, vascularização tumoral, interações com o microambiente, infiltração para vasos sanguíneos ou linfáticos, e sobrevivência celular em sítios distantes (HANAHAN; WEINBERG, 2000). Dentre os diversos fatores envolvidos, a perda de Caderina E tem sido relacionada à capacidade de invasão tumoral e desenvolvimento de metástases, inclusive em modelos de carcinomas mamários murinos (VLEMINCKX et al., 1991).

Além da carga metastática, outro importante parâmetro correlacionado à agressividade do tumor de mama e, portanto, ao prognóstico das pacientes, é a taxa de crescimento do tumor primário (CARTER; ALLEN; HENSON, 1989). Assim, observações histológicas e/ou análises de marcadores séricos ou tumorais tem sido complementados com a avaliação de fatores anatômicos (como tamanho do tumor primário, presença de metástases em linfonodos regionais, presença de metástases distantes), representada pelo sistema de estadiamento tumor-linfonodo-metástase (TNM) (SINGLETARY et al., 2002).

### 2.1.2 Modelos experimentais de carcinogênese mamária

Modelos experimentais são ferramentas úteis para se avaliar o desenvolvimento neoplásico. O estudo de neoplasias em modelos animais facilita a avaliação e melhor compreensão da biologia dos tumores, ao mesmo tempo em que constitui instrumento apropriado para a investigação de efeitos anti-inflamatório, anti-angiogênico e anti-proliferativo de substâncias com potencial antineoplásico. Um desses modelos é o carcinoma mamário 4T1. Trata-se de uma linhagem celular altamente tumorigênica e invasiva, onde são observadas metástases em diversos órgãos, incluindo linfonodos, fígado, pulmão e cérebro após duas semanas de inoculação subcutânea das células (PULASKI; OSTRAND-ROSENBERG, 2001).

O modelo animal 4T1 foi inicialmente descrito por Fred Miller e colaboradores (DEXTER et al., 1978; ASLAKSON; MILLER, 1992). As células cancerosas podem ser inoculadas em camundongos *Balb/c*, gerando tecidos tumorais que são resistentes a 6-thioguanina. O modelo experimental 4T1 apresenta várias características que o fazem um excelente modelo para estudo da carcinogênese mamária humana: as células cancerosas

podem ser inoculadas no sítio primário (mama) ou em área ectópica (como no flanco, por exemplo) e, em ambos os casos, originam metástases espontaneamente. O sítio primário pode ser removido cirurgicamente e os sítios metastáticos podem ser estudados intactos e isoladamente. Além disso, os tumores podem ser manipulados facilmente *in vivo* ou *in vitro* (PULASKI; OSTRAND-ROSENBERG, 1998; PULASKI; OSTRAND-ROSENBERG, 2001; BALIGA; MELETH; KATIYAR, 2005).

Morfologicamente, o carcinoma mamário 4T1 apresenta proliferação epitelial maligna em arranjo sólido, caracterizado pela proliferação de células pleomórficas e elevado índice mitótico. Diferentes estudos terapêuticos são descritos com a utilização desse modelo na caracterização de eventos celulares e moleculares durante a progressão neoplásica, sugerindo sua utilização como modelo experimental de grande relevância clínica em seres humanos (PULASKI; OSTRAND-ROSENBERG, 2001; BALIGA; MELETH; KATIYAR, 2005).

## 2.2 Selênio (Se)

Define-se micronutriente como qualquer elemento nutricional necessário em quantidades mínimas, tais como as vitaminas e os minerais. Pode também ser definido como qualquer substância dietética, essencial ou não essencial, que está presente em pequenos valores e traz um efeito fisiológico. Há relatos do uso de micronutrientes em ensaios clínicos de quimioprevenção, com dados epidemiológicos e estudos pré-clínicos em animais e culturas de células (GREENWALD et al., 2002; LI et al., 2004).

O Se é um micronutriente relacionado a diversos benefícios para a saúde humana e de outros mamíferos, tais como a redução da incidência de câncer (IP; MEDINA, 1987; MEDINA; MORRISON, 1988; CLARK et al., 1996; CLARK et al., 1998; LI et al., 2004; RAYMAN, 2005; ALMONDES et al., 2010; HATFIELD et al., 2012; CHEN; PRABHU; MASTRO, 2013), bem como da mortalidade dos pacientes oncológicos e por causas gerais (CLARK et al., 1996; CLARK et al., 1998; AKBARALY et al., 2005; RAY et al., 2006; BLEYS; NAVAS-ACIEN; GUALLAR, 2008; RAYMAN, 2012), além de proteção contra disfunções da tireóide (OLIVIERI et al., 1995; NEGRO et al., 2007; RAYMAN, 2012), manutenção da saúde reprodutiva (UNDERWOOD, 1977; HIDIROGLOU, 1979; BEHNE; WEILER; KYRIAKOPOULOS, 1996; RAYMAN, 2012) e neurológica (SCHWEIZER et al., 2004; ASHRAFI et al., 2007a; ASHRAFI et al., 2007b; BURK; HILL, 2009; SHAHAR et al., 2010; TAKEMOTO; BERRY; BELLINGER, 2010), proteção contra doenças cardiovasculares (SALONEN et al., 1982; FLORES-MATEO et al., 2006; NAVAS-ACIEN;

BLEYS; GUALLAR, 2008; RAYMAN, 2012) e contra desordens musculares (WHANGER, 2000; ZORZATO et al., 2007), e atraso no início dos sintomas da Síndrome da Imunodeficiência Adquirida (AIDS) em pacientes soropositivos para o vírus HIV (SAPPEY et al., 1994; BAUM et al., 1997; CAMPA et al., 1999; RAYMAN, 2000). Esses efeitos se devem principalmente às suas ações antioxidantes, neuroprotetoras, de regulação da produção de citocinas pró-inflamatórias e da biossíntese de leucotrienos e tromboxanos, dentre outros mecanismos (BRIGELIUS-FLOHE et al., 1994; BRIGELIUS-FLOHE, 1999; KOHRL et al., 2000; RAYMAN, 2000; URSINI, 2000; UNNI et al., 2001; IMAI et al., 2003; RAYMAN, 2005; UNNI et al., 2005; PAPP et al., 2007; RAYMAN, 2009; STEINBRENNER; SIES, 2009; KASAIKINA; HATFIELD; GLADYSHEV, 2012; KIPP et al., 2012; RAYMAN, 2012; BRIGELIUS-FLOHE; MAIORINO, 2013; KUMAR; PRIYADARSINI, 2014; LABUNSKYY; HATFIELD; GLADYSHEV, 2014; TOUAT-HAMICI et al., 2014; TSUJI et al., 2015; DKHIL et al., 2016; STEINBRENNER; SPECKMANN; KLOTZ, 2016; WROBEL; POWER; TOBOREK, 2016; HOSNEDLOVA et al., 2017; SOLOVYEV et al., 2018).

Considerado inicialmente como tóxico quando ingerido em altas concentrações (FRANKE; PAINTER, 1938; MOXON; RHIAN, 1943; RAYMAN, 2008), o Se só foi reconhecido como um mineral essencial a partir de 1957 ao ser demonstrada sua função preventiva contra necrose hepática em ratos (SCHWARZ; FOLTZ, 1957). A associação entre baixo status de Se e risco aumentado na mortalidade e/ou incidência de câncer, sugerida pela primeira vez no final dos anos 1960 (SHAMBERGER; FROST, 1969; SCHRAUZER; RHEAD, 1971; SHAMBERGER; WILLIS, 1971; WILLETT et al., 1983; CLARK et al., 1984, 1993; FORDYCE, 2007), atraiu tanta atenção que centenas de milhares de dólares foram investidos em triagens clínicas examinando seu efeito quimiopreventivo (CLARK et al., 1996; MCCLAIN, 2002; NATIONAL CANCER INSTITUTE, 2005; LIPPMAN et al., 2009; DUNN; TAYLOR, 2012). Porém, apesar de alguns resultados positivos (CLARK et al., 1996; YOSHIZAWA et al., 1998), em outras triagens nenhum efeito benéfico foi detectado (NATIONAL CANCER INSTITUTE, 2005; LIPPMAN et al., 2009), levando inclusive ao encerramento precoce de uma das maiores triagens preventivas contra câncer já conduzidas (LIPPMAN et al., 2009). Atualmente sabe-se que estudos epidemiológicos demonstram que existe uma associação inversa entre concentração sérica de selênio e risco de mortalidade, contudo essa associação foi observada apenas em indivíduos com até 130 µg Se/L sangue, enquanto que um leve aumento do risco de mortalidade foi relatado em concentrações acima de 150 µg/L (KIPP et al., 2015).

O Se é um elemento-traço que pode se apresentar na forma inorgânica, metálica ($Se^0$) ou oxiânions como selenito ($SeO(OH)_2$) e selenato ($SeO_2(OH)_2$), e também na forma orgânica, como selenocisteína (SeCys) e selenometionina (SeMet) - análogos dos aminoácidos sulfurados cisteína e metionina, respectivamente (SUZUKI, 2005; SURAI, 2006; ALMONDES et al., 2010). No organismo animal, as proteínas contendo Se na forma de SeCys são chamadas de selenoproteínas, enquanto aquelas que contêm Se na forma de SeMet são chamadas simplesmente de proteínas contendo Se (SUZUKI, 2005; MEHDI et al., 2013; BURK; HILL, 2015). Em seres humanos, já foram descritos 25 genes que codificam as selenoproteínas, ao passo que nos roedores foram descritos 24 (KRYUKOV et al., 2003; ZHANG et al., 2008).

A principal fonte de Se para humanos e outros mamíferos se dá pela dieta (RAYMAN, 2008) e os sintomas de sua deficiência ou de suas alterações genotípicas incluem infertilidade masculina (BURK; HILL, 2009), doença de Keshan (COMBS; COMBS, 1986), doença de Kashin-Beck (COMBS; COMBS, 1986), redução da resposta imune (CARLSON et al., 2010; HOFFMANN et al., 2010), declínio cognitivo (BERR et al., 2000; AKBARALY et al., 2007; GAO et al., 2007), e aumento da incidência de diferentes tipos de cânceres, como de próstata (WILLETT et al., 1983; CLARK et al., 1996; CLARK et al., 1998; ETMINAN et al., 2005; BRINKMAN et al., 2006; PETERS; TAKATA, 2008; GILL et al., 2009; RAYMAN, 2010), cólon (CLARK et al., 1996; DAVIS; UTHUS; FINLEY, 2000; PETERS; TAKATA, 2008), pulmão (CLARK et al., 1996; ZHUO; SMITH; STEINMAUS, 2004; MAHABIR et al., 2006), fígado (YU et al., 1999; DAVIS; UTHUS; FINLEY, 2000), bexiga (AMARAL et al., 2010; RAYMAN, 2009), tireóide (GLATTRE et al., 1989) e de mama (HUNTER et al., 1990; HARDELL et al., 1993; RAYMAN, 2005; REJALI; JAAFAR; ISMAIL, 2007; HARRIS; BERGKVIST; WOLK, 2012; CAI et al., 2016), e de outras doenças crônicas não transmissíveis (doenças cardiovasculares e diabetes mellitus, por exemplo) (SALONEN et al., 1982; RAJPATHAK et al., 2005; LOH et al., 2009; RAYMAN, 2012; KUMAR; PRIYADARSINI, 2014).

Dentre as diversas funções biológicas conhecidas, sabe-se que o Se atua também como um agente antimutagênico (FERGUSON et al., 2012), prevenindo danos em DNA que poderiam levar a transformações malignas de células normais (KARUNASINGHE et al., 2004; KOWALSKA et al., 2005; WATERS et al., 2005). Contudo, estudos mostram que os efeitos anticâncer do Se envolvem múltiplos mecanismos, incluindo ação antioxidante, atividade imunoestimulatória, regulação de enzimas conjugadoras de fase II, alteração da metilação do DNA, indução de apoptose de células tumorais, inibição de angiogênese

tumoral, e modulação da via Wnt, do fator de crescimento endotelial vascular (VEGF) plasmático e da proteína supressora de tumor p53, dentre outros (IP; LISK, 1997; JIANG et al., 1999; DAVIS; UTHUS; FINLEY, 2000; UNNI et al., 2001; DONG et al., 2002; DAVIS; FINLEY, 2003; DAVIS; UTHUS, 2003; SMITH et al., 2004; RAYMAN, 2005; UNNI et al., 2005; HOFFMANN, 2007; BRIGELIUS-FLOHE, 2008; BRIGELIUS-FLOHE; KIPP, 2009; KIPP et al., 2009; KIPP et al., 2012; BARRETT et al., 2012; FERGUSON et al., 2012; RESZKA, 2012; SANMARTIN et al., 2012; BRIGELIUS-FLOHE; KIPP, 2013; GUO; HSIA; CHEN, 2013; MEHDI et al., 2013; MISRA et al., 2014; WALLENBERG et al., 2014; WALLENBERG; MISRA; BJORNSTEDT, 2014; FAGHFURI et al., 2015; FERNANDES; GANDIN, 2015; TOBE et al., 2015; TSUJI et al., 2015; EKOUE et al., 2017). Apesar dos vários mecanismos de ação existentes, este efeito protetor do Se foi primeiramente associado à sua presença na glutationa peroxidase (GPx) e na tioredoxina redutase (TrxR), enzimas que são conhecidas por proteger o DNA e outros componentes celulares contra o dano oxidativo (KIPP et al., 2009; KIPP et al., 2012).

### 2.2.1 Glutationa Peroxidase

A família conhecida como GPx inclui diversas enzimas Se-dependentes que diferem quanto ao peso molecular, substrato específico, distribuição tissular, gene codificador e função (URSINI; MAIORINO; ROVERI, 1997; CHENG et al., 1998; BRIGELIUS-FLOHE, 1999; SURAI, 2006). Atualmente, são conhecidas cinco enzimas GPx humanas que contêm um átomo de selênio na forma de SeCys. São elas: GPx-1 que é encontrada no citoplasma e no interior de hemácias; GPx-2, uma enzima encontrada no trato gastrointestinal; GPx-3 que está presente no plasma; GPx-4 que atua em lipídios oxidados, e é chamada de glutationa peroxidase fosfolipídio hidroperóxido; e a GPx-sn que é uma enzima específica do núcleo de espermas. Todas estas enzimas GPx têm em comum uma tríade catalítica no seu centro ativo consistindo de SeCys, glutamina e resíduos de triptofano. Sua ação antioxidante é devido à redução de peróxidos de hidrogênio, hidroperóxidos orgânicos e fosfolipídio hidroperóxido (somente a GPx-4) (KIPP et al., 2009; STEINBRENNER; SIES, 2009; KIPP et al., 2012).

Alguns fatores afetam, em níveis diferentes, a atividade e a expressão das enzimas desse grupo (ALLAN; LACOURCIERE; STADTMAN, 1999; SUNDE, 2006; SUNDE et al., 2009; ROMAN; JITARU; BARBANTE, 2014). Por exemplo, a hepatotoxina nodularin, conhecida por promover tumores, causa decréscimo dose-dependente da atividade da GPx-1 em fígado de camundongos (LANKOFF; BANASIK; NOWAK, 2002) e já foi demonstrado

que, quanto maior o estresse oxidativo, menor a atividade dessa enzima em eritrócitos de ratos (GABBIANELLI et al., 2002; SURAI, 2006). Além disso, dentre os membros da família GPx, a GPx-1 é considerada a mais sensível a mudanças tanto do status de Se quanto a situações de estresse oxidativo (SUNDE et al., 2009; ROMAN; JITARU; BARBANTE, 2014), apesar desta mostrar rápida recuperação em comparação a outras selenoproteínas (PAPP et al., 2007).

A atividade antioxidante é considerada um fator de proteção contra câncer (RAYMAN, 2005; GOH et al., 2011; SOTGIA; MARTINEZ-OUTSCHOORN; LISANTI, 2011; GUO; HSIA; CHEN, 2013), contudo trabalhos sugerem que os diferentes tipos de GPx possuem diferentes papéis de acordo com o tipo de câncer e da fase carcinogênica (BRIGELIUS-FLOHE; KIPP, 2009; RAYMAN, 2009; BRIGELIUS-FLOHE; MAIORINO, 2013; TOBE et al., 2015). No caso da GPx-1, um levantamento que avaliou genótipo de mulheres com e sem câncer de mama, relatou que a perda de heterozigose em GPx-1 foi observada em pacientes com tumor, implicando na possibilidade de envolvimento desta GPx no desenvolvimento desse tipo de câncer (HU; DIAMOND, 2003).

### 2.2.2 Selenoproteína P

Após absorção intestinal do Se, a regulação deste mineral ocorre no fígado antes de ser levado aos demais tecidos do corpo (BURK; HILL; MOTLEY, 2003; ROMAN; JITARU; BARBANTE, 2014). Para este transporte é essencial que o fígado sintetize a selenoproteína P (SelP), permitindo que o Se chegue a vários tecidos (BURK et al., 2006; PAPP et al., 2007), principalmente cérebro e testículos (HILL et al., 2003). Estudos realizados com camundongos *knock-out* para SelP demonstram que esta selenoproteína é absolutamente requerida pelo cérebro para evitar disfunção neurológica (HILL et al., 2004) e degeneração axonal do tronco cerebral (VALENTINE et al., 2005). A proteção contra a oxidação por peroxinitrito também é uma de suas ações relatadas (ARTEEL et al., 1998), bem como sua ação antioxidante no espaço extracelular (BURK; HILL, 2005).

Normalmente, a SelP é altamente expressa pelo epitélio prostático, mas sofre *down regulation* em tumores humanos de próstata, tumores murinos e linhagens de células de carcinoma prostático (CALVO et al., 2002). Em mucosa de cólon normal, o gene humano da SelP também é abundantemente expresso, ocorrendo redução significativa ou perda de expressão de RNA mensageiro (mRNA) desta selenoproteína em cânceres de cólon (AL-TAIE et al., 2004). Além disso, foi observado, em pesquisa recente, que o aumento de sua

expressão promoveu proteção de fibroblastos humanos normais contra toxicidade induzida por radiação (ECKERS et al., 2013).

Em recente revisão para cálculo dos valores de referência de ingestão diária de Se, foi sugerida que a avaliação de SelP seria mais eficaz do que a avaliação da GPx (KIPP et al., 2015). Isso se deve ao fato de que a associação inversa entre concentração sérica de Se e risco de mortalidade em humanos foi detectada apenas em indivíduos apresentando cerca de 130 µg Se/L sangue (BLEYS; NAVAS-ACIEN; GUALLAR, 2008). Como a atividade ótima da GPx plasmática ocorre quando se tem níveis de até 90 µg Se/L sangue (XIA et al., 2005; XIA et al., 2010), a saturação desta impediria a comparação entre grupos experimentais com alto teor de Se. Portanto, em pesquisas oncológicas utilizando doses suplementares de Se, a análise de SelP tem se mostrado como importante ferramenta. É importante considerar que níveis acima de 150 µg Se/L sangue têm sido associados a um leve aumento no risco de morte de pacientes de câncer (BLEYS; NAVAS-ACIEN; GUALLAR, 2008), o que instiga novas pesquisas visando esclarecer a estreita faixa entre a essencialidade e a toxicidade deste mineral tão controverso.

### 2.2.3 Selênio e alimentação

A importância da dieta no desenvolvimento e progressão de câncer foi inicialmente sugerida através de estudos epidemiológicos quando se estimou que 35% das mortes por câncer nos Estados Unidos atribuíam-se à dieta, sem nenhuma referência específica à obesidade ou ao sedentarismo (DOLL; PETO, 1981; DOLL, 1992; DONALDSON, 2004; SURAI, 2006). Sendo assim, a inclusão de antioxidantes, como o Se, na dieta representaria uma importante ferramenta preventiva contra o câncer (ZAIDI; BANU, 2004; SOTGIA; MARTINEZ-OUTSCHOORN; LISANTI, 2011).

Estudos sugerem que o processo carcinogênico pode ser prevenido pela suplementação de Se tanto na fase inicial quanto na fase de progressão, mesmo que essa suplementação seja realizada por curto período de tempo englobando a fase de gênese tumoral (BJORKHEM-BERGMAN et al., 2005; SURAI, 2006; CHEN et al., 2013). Contudo, há relato de que os efeitos benéficos mais significativos do Se foram promovidos a longo prazo contínuo e/ou antes da fase inicial da carcinogênese, ao invés da fase de progressão (THIRUNAVUKKARASU; SAKTHISEKARAN, 2003), o que motiva a realização de pesquisas que desvendem a melhor forma de utilização desse mineral através da alimentação.

O Se pode ser encontrado em cereais, castanhas, carnes, ovos, cogumelos, vegetais (especialmente alho, cebola e brócolis) e em alguns frutos do mar, dentre outros alimentos, e a recomendação atual é de que um humano adulto consuma, no mínimo, de 60 a 70 μg de Se por dia (INSTITUTE OF MEDICINE, 2000; KIPP et al., 2015). Sabe-se que a biodisponibilidade do Se é variável de acordo com as diferentes fontes, o status de Se do indivíduo, sua condição fisiológica, a espécie animal, os demais componentes da dieta, dentre outros fatores (ULLREY, 1987; BATES et al., 2002; SURAI, 2006).

Considerada como um dos alimentos mais ricos em Se, a castanha do Brasil (*Bertholletia excelsa*) apresenta valores que podem variar entre 0,2 e 512 μg/g, estando este presente principalmente nas formas de SeMet e SeCys (CHANG et al., 1995; FERREIRA et al., 2002; VONDERHEIDE et al., 2002; PACHECO; SCUSSEL, 2007; FREITAS et al., 2008; PAREKH et al., 2008; SILVA JUNIOR et al., 2017). Trata-se de um alimento consumido mundialmente e que possui cerca de 15% de proteínas, 9% de carboidratos e 71% de lipídeos (STOCKLER-PINTO et al., 2015). A presença de vitamina E e de altos níveis de ácidos graxos mono e poli-insaturados em sua composição são outros fatores que lhe dão grande destaque nutricional (CHANG et al., 1995; DUMONT et al., 2006; ROS; MATAIX, 2006; YANG, 2009).

O consumo desta castanha por pessoas obesas é capaz de reduzir riscos associados a doenças cardiovasculares através da melhora do status de Se e do perfil lipídico (COMINETTI et al., 2012). Além disso, seu consumo foi capaz de aumentar a atividade de GPx e o perfil de hormônios da tireóide, demonstrando efeito anti-inflamatório e antioxidante em pacientes de hemodiálise (STOCKLER-PINTO et al., 2014, 2015). Detectou-se aumento de Se plasmático, Se eritrocitário e atividade de GPx após ingestão ao longo de 8 semanas por mulheres obesas que inicialmente eram deficientes em Se (COMINETTI et al., 2011).

O Se atua em estreita relação com outros antioxidantes, principalmente com a vitamina E (LEVANDER; AGER; BECK, 1995; SURAI 2006). Sabe-se que a interação entre Se e vitamina E permitiria um aumento da capacidade antioxidante do organismo, visto que a proteção fornecida pela GPx contra a oxidação dos ácidos graxos presentes nas membranas celulares permite uma redução da quantidade de vitamina E requerida para manutenção dessa integridade (SURAI, 2003). Além disso alguns estudos demonstram que estes dois nutrientes podem agir sinergisticamente na prevenção de alguns tipos de câncer (HORVATH; IP, 1983; IP; WHITE, 1987; KLEIN et al. 2000).

Na Finlândia, na década de 70, a população apresentava baixos níveis séricos de Se e a incidência de doenças cardiovasculares estava entre as mais altas do mundo, e a hipótese

levantada pelos pesquisadores foi a de que a deficiência de Se poderia ser uma das razões. Nessa época, foi iniciado um experimento em grande escala acrescentando Se nos fertilizantes, aumentando o teor desse micronutriente nos alimentos locais e dobrando, assim, a concentração de Se sérico na população, o que reduziu a incidência das doenças cardiovasculares e de câncer (PIETINEN et al., 1996; ALFTHAN, 2005; COMBS JR., 2005; STEINNES, 2009). Portanto, uma possível estratégia para diminuir a deficiência de Se na população mundial poderia ser o aumento do consumo rotineiro de alimentos que contenham naturalmente maiores concentrações de Se, como a castanha do Brasil (FREITAS et al., 2008). Sendo assim, estudos que avaliem os efeitos dessa ingestão poderiam prevenir morbidades, de modo a reduzir gastos com tratamentos de saúde.

### 2.2.4 Absorção e metabolismo de selênio

Dados da literatura sugerem que Se orgânico em forma de diversos selenoaminoácidos, principalmente como SeMet, representa uma forma natural do Se na dieta de humanos e de outros animais, e que o sistema digestivo se adaptou a essa forma nutricional ao longo da evolução, o que explicaria as diferenças na assimilação e metabolismo dentre as formas orgânicas e inorgânicas de Se (COMBS; COMBS, 1986; SCHRAUZER, 2000; SURAI 2002; SCHRAUZER, 2003).

A SeMet é absorvida de maneira semelhante à metionina (FAIRWEATHER-TAIT, 1997), através de transporte ativo com carreadores presentes nas membranas dos enterócitos ao longo de todo o intestino, sendo esta absorção maior no duodeno e decrescida nos segmentos posteriores (WOLFFRAM et al., 1989; ANDERSEN et al., 1994). A SeCys é absorvida de maneira análoga ao aminoácido cisteína e, portanto, também depende do transporte ativo para alcançar a circulação sanguínea. Sendo assim, sabendo-se que o sistema de transporte ativo intestinal está sujeito a uma autorregulação em condições de excesso do nutriente, é de se considerar a possibilidade de saturação desse transporte em organismos com alto status de Se que estejam ingerindo Se em doses suplementares (MANIS; SCHACHTER, 1964), o que explicaria a melhor resposta à suplementação em indivíduos deficientes em Se (THOMSON, 1998; RAYMAN, 2005; SURAI, 2006; LEE et al., 2011). Em contraste, selenato é absorvido paracelularmente através de processo de difusão passiva, enquanto selenito pode ter sua absorção aumentada pela ação da glutationa reduzida presente no fluido gastrointestinal e sofrer mais transformações dos que os demais compostos de Se antes de alcançar a circulação (WHANGER et al., 1996; SUZUKI, 2005; GAMMELGAARD et al.,

2012). Apesar de não serem conhecidas as proteínas de transporte envolvidas na absorção direta e indireta de selenito (ROMAN; JITARU; BARBANTE, 2014), sabe-se que as formas inorgânicas são melhor absorvidas no íleo (ANDERSEN et al., 1994).

Após absorção intestinal, íons de selenito são imediatamente capturados pelas hemácias e reduzidos a selenido, para então serem liberados de volta à circulação e carreados pela albumina até o fígado, onde serão utilizados para formação de SelP e GPx-1 hepática. Já íons de selenato circulam livremente pelo sangue até chegar aos hepatócitos, para então também serem utilizados para síntese de SelP e GPx-1 hepática, ou então serem eliminados pela urina (SUZUKI; OGRA, 2002; SUZUKI, 2005). Ainda não estão completamente esclarecidos os processos que ocorrem com as formas orgânicas antes de sua chegada ao fígado, contudo é possível que a SeMet seja incorporada de modo inespecífico a proteínas como albumina e hemoglobina (Hb) (SUZUKI; OGRA, 2002).

A rápida redução das formas inorgânicas a selenido para posterior utilização e/ou excreção como metabólitos metilados (SUZUKI, 2005) poderia explicar a maior toxidez destas (HERIGSTAD; WHITEHAIR; OLSON, 1973; BORELLA; BARGELLINI; MEDICI, 1996; SCHRAUZER, 2003), visto que as formas orgânicas sofrem essa transformação de maneira mais complexa através de reações de lise (ESAKI et al., 1981; ESAKI et al., 1982; GANTHER, 1986; SCHRAUZER, 2003; SCHOMBURG; SCHWEIZER; KOHRLE, 2004). A SeCys necessita ser lisada a selenido através da enzima beta-liase, enquanto a SeMet deve ser convertida, pela via trans-selenação, a Se-adenosil-SeMet, Se-adenosilselenohomocisteína, selenohomocisteína, selenocistationina e SeCys para só então ser lisada a selenido (SUZUKI, 2005). Além disso, é importante considerar a toxidez do excesso de qualquer antioxidante, que acaba por se tornar pró-oxidante nessas condições (SURAI et al., 1998; SURAI, 2000; SURAI, 2006).

O fígado é o principal órgão relacionado ao metabolismo de Se, sendo, portanto, algumas vezes utilizado em experimentos para indicar o status de Se corporal através da quantificação de Se e/ou da expressão ou atividade da GPx-1 (RIESE et al., 2006; LEI; CHENG; MCCLUNG, 2007; KIPP et al., 2009; CHEN et al., 2013; GUO; HSIA; CHEN, 2013). A SelP sintetizada no fígado permitirá o transporte de Se para os demais tecidos para que estes sintetizem suas respectivas selenoproteínas de acordo com suas necessidades (BURK; HILL, 2015).

Enquanto a SeMet pode ser acumulada de maneira inespecífica em tecidos como fígado e músculos (SHIOBARA; SUZUKI, 1998; SCHRAUZER, 2003), não há relatos de detecção de SeCys livre em homogenatos de tecidos, sugerindo que sua concentração em

tecidos seja muito baixa (ESAKI et al., 1981; BURK; HILL, 2015). As formas inorgânicas também são pouco depositadas no organismo, sendo seu excesso eliminado pela urina, fezes e respiração através do processo de metilação (MCCONNELL; ROTH, 1966; WOLFFRAM, 1999).

Somente após a conversão a selenido a partir dos diferentes compostos de Se é que são sintetizadas as selenoproteínas, sendo o selenido, portanto, o metabólito intermediário comum a todos estes (SUZUKI; OGRA, 2002). Estudos oncológicos têm dado especial atenção ao metabólito metilselenol, visto que tem se sugerido a sua participação em funções fisiológicas anticarcinogênicas quando este se deriva de SeMet ou de Se-metilselenocisteína (IP et al., 2000; DONG et al., 2001; IP; DONG; GANTHER, 2002; SEO; KELLEY; SMITH, 2002; DONG et al., 2003).

Em amostras de plasma humano, cerca de 60% do Se se encontra como SelP e 3% em forma de GPx-3, havendo diferenças nesses valores quando comparados a outras espécies animais (SUZUKI; SAKAI; FURUTA, 2012). Em sangue total, além dessas selenoproteínas há ainda Se depositado em GPx-1 eritrocitária e menores concentrações em forma de SeMet, íon trimetilselenônio e selenoaçúcar em hemácias (COMBS, 2015). Contudo, é possível que as espécies de Se presentes no sangue dependam da fonte dietética de Se (SURAI, 2006). Tem-se relato de que mulheres ingerindo SeMet em dose suplementar apresentaram a maior parte do Se sanguíneo contido na Hb, enquanto o Se sanguíneo foi igualmente distribuído entre GPx e Hb em mulheres ingerindo selenato (BUTLER et al., 1991). Similarmente, ratos alimentados com selenito ou SeCys apresentaram a maioria do Se eritrocitário na forma de GPx, enquanto os ratos que ingeriram SeMet, levedura ou trigo apresentaram mais Se depositado na Hb do que na GPx (BEILSTEIN; WHANGER, 1986). Além disso, foi demonstrado que Se oriundo de SeMet pode ser incorporado à albumina, ao contrário de selenato e SeCys (BURK; HILL; MOTLEY, 2001). Assim, a avaliação do efeito específico das diversas fontes de Se é necessária para compreender qual(is) selenoproteína(s) é(são) mais estimulada(s) em cada situação.

Para avaliação do status de Se, não existe um teste único, sendo ideal a combinação de diversas técnicas (SURAI, 2006). Os chamados testes estáticos incluem análises da quantidade total de Se em unha, cabelo, sangue (total ou de seus componentes) ou urina. Já os testes funcionais avaliam a atividade de enzimas Se-dependentes ou as funções fisiológicas dependentes de Se (GIBSON, 1989). A concentração de SelP reflete um status de curto prazo (BURK; HILL, 2005), assim como o nível de Se em plasma ou soro. Já a concentração de Se em eritrócitos reflete um status de longo prazo, assim como a quantificação em cabelo e unha

(THOMSON, 2004). É válido ressaltar que polimorfismos genéticos, idade, sexo, grupo étnico, dentre outros fatores interferem na resposta dos diferentes biomarcadores (BATES et al., 2002; COMINETTI et al., 2011). Todos esses fatores influenciadores, bem como as análises estatísticas utilizadas, as técnicas laboratoriais de quantificação e a conservação das amostras podem ser possíveis explicações para os resultados controversos presentes na literatura (SURAI, 2006).

## 3 CONSIDERAÇÕES FINAIS

A relação entre Se dietético e diversos tipos de câncer tem sido estudada há cerca de quatro décadas, porém os resultados controversos e/ou inconclusivos demonstram a necessidade de mais pesquisas a respeito, de modo a trazer informações mais aplicáveis para a saúde da população.

A castanha do Brasil é reconhecida mundialmente por seu potencial como rica fonte de Se, porém ainda são escassos os trabalhos que tenham avaliado seu efeito sobre a progressão de tumor mamário. A suplementação através de fontes como a SeMet pode ser uma importante ferramenta visto que, em diversas pesquisas, mostrou-se como opção eficiente tanto para a prevenção quanto para a redução da progressão tumoral, o que motiva a execução de mais experimentos que avaliem seu efeito.

O consumo de suplementos com Se deve ser avaliado com cautela, uma vez que a biodisponibilidade do nutriente depende de diversos fatores, tais como a fonte de Se, espécie animal, presença de outros nutrientes no intestino, status de Se no organismo, entre outros. Adicionalmente, deve-se considerar que a faixa terapêutica do Se é relativamente estreita, em que doses elevadas podem apresentar efeitos adversos como, por exemplo, um maior número de metástases em pacientes oncológicos.

# REFERÊNCIAS


AKBARALY, N. T. et al. Selenium and mortality in the elderly: results from the EVA study. **Clinical Chemistry**, v. 51, n. 11, p. 2117-2123, Nov. 2005.

AKBARALY, N. T. et al. Plasma selenium over time and cognitive decline in the elderly. **Epidemiology**, v. 18, n. 1, p. 52-58, Jan. 2007.

ALLAN, C. B.; LACOURCIERE, G. M.; STADTMAN, T. C. Responsiveness of selenoprotein to dietary selenium. **Annual Review of Nutrition**, v. 19, p. 1-16, 1999.

ALFTHAN, G. Trends in blood and tissue selenium levels in Finland 1984-2004. In: Proceedings Twenty Years of Selenium Fertilization (Merja Eurola ed.), Helsinki, Finland. **Agrifood Research Reports**, v.69, p.71-76. 2005.

ALMONDES, K. G. et al. The role of selenoproteins in cancer. **Revista da Associação Médica Brasileira,** v. 56, n. 4, p. 484-488, Jul./Aug. 2010.

AL-TAIE, O. H. et al. Expression profiling and genetic alterations of the selenoproteins GI-GPx and SePP in colorectal carcinogenesis. **Nutritional and Cancer,** v. 48, n. 1, p. 6-14, 2004.

AMARAL, A. F. S. et al. Selenium and bladder cancer risk: a meta-analysis. **Cancer Epidemiology Biomarkers & Prevention**, v. 19, n. 9, p. 2407-2415, Aug. 2010.

AMBROSONE, C.B. et al. Manganese superoxide dismutase (MnSOD) genetic polymorphisms, dietary antioxidants, and risk of breast cancer. **Cancer Research**, v. 59, n. 3, p. 602-606, Feb. 1999.

ANDERSEN, O. et al. Experimental localization of intestinal uptake sites for metals (Cd, Hg, Zn, Se) in vivo in mice. **Environmental Health Perspectives,** v. 102, n. Suppl. 3, p. 199-206, Sep. 1994.

ARTEEL, G. E. et al. Protection by selenoprotein P in human plasma against peroxynitrite-mediated oxidation and nitration. **Biological Chemistry,** v. 379, n. 8-9, p. 1201-1205, Aug./Sep. 1998.

ASHRAFI, M. R. et al. Selenium and intractable epilepsy: is there any correlation? **Pediatric Neurology**, v. 36, n. 1, p. 25-29, Jan. 2007a.

ASHRAFI, M. R. et al. A probable causative factor for an old problem: selenium and glutathione peroxidase appear to play important roles in epilepsy pathogenesis. **Epilepsia**, v. 48, n. 9, p. 1750-1755, Sep. 2007b.

ASLAKSON, C. J.; MILLER, F. R. Selective events in the metastatic process defined by analysis of the sequential dissemination of subpopulations of a mouse mammary tumor. **Cancer Research,** v. 52, n. 6, p. 1399-1405, Mar. 1992.

BAI, R-K. et al. Mitochondrial genetic background modifies breast cancer risk. **Cancer Research**, v. 67, n. 10, p. 4687-4694, May 2007.

BALIGA, M. S.; MELETH, S.; KATIYAR, S. K. Growth inhibitory and antimetastatic effect of green tea polyphenols on metastasis-specific mouse mammary carcinoma 4t1 cells in vitro and in vivo systems. **Clinical Cancer Research,** v. 11, n. 5, p. 1918-1927, Mar. 2005.

BARRETT, C. W. et al. Tumor suppressor function of the plasma glutathione peroxidase Gpx3 in colitis-associated carcinoma. **Cancer Research**, v. 73, n. 3, p. 1245-1255, Dec. 2012.

BATES, C. J. et al. Selenium status and associated factors in a British National Diet and Nutrition Survey: young people aged 4-18 y. **European Journal of Clinical Nutrition,** v. 56, n. 9, p. 873-881, Sep. 2002.

BAUM, M. K. et al. High risk of HIV-related mortality is associated with selenium deficiency. **JAIDS Journal of Acquired Immune Deficiency Syndromes**, v. 15, n. 5, p. 370-374, Aug. 1997.

BEHNE, D.; WEILER, H.; KYRIAKOPOULOS, A. Effects of selenium deficiency on testicular morphology and function in rats. **Journal of Reproduction and Fertility**, v. 106, n. 2, p. 291-297, Mar. 1996.

BEILSTEIN, M. A.; WHANGER, P. D. Deposition of dietary organic and inorganic selenium in rat erythrocyte proteins. **The Journal of Nutrition,** v. 116, n. 9, p. 1701-1710, Sep. 1986.

BENHAR, M.; ENGELBERG, D.; LEVITZKI, A. ROS, stress-activated kinases and stress signaling in cancer. **EMBO Reports**, v. 3, n. 5, p. 420-425. May 2002.

BERR, C. et al. Cognitive decline is associated with systemic oxidative stress: the EVA study. **Journal of the American Geriatrics Society**, v. 48, n. 10, p. 1285-1291, Oct. 2000.

BJÖRKHEM-BERGMAN, L. et al. Selenium prevents tumor development in a rat model for chemical carcinogenesis. **Carcinogenesis,** v. 26, n. 1, p. 125-131, Jan. 2005.

BLEYS, J.; NAVAS-ACIEN, A.; GUALLAR, E. Serum selenium levels and all-cause, cancer, and cardiovascular mortality among US adults. **Archives of Internal Medicine,** v. 168, n. 4, p. 404-410, Feb. 2008.

BORELLA, P.; BARGELLINI, A.; MEDICI, C. I. Chemical form of selenium greatly affects metal uptake and response by cultured human lymphocytes. **Biological Trace Element Research**, v. 51, n. 1, p. 43-54, Jan. 1996.

BRANDON, M.; BALDI, P.; WALLACE, D. C. Mitochondrial mutations in cancer. **Oncogene**, v. 25, n. 34, p. 4647-4662, Aug. 2006.

BRAY, F. et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. **CA: A Cancer Journal for Clinicians**, v. 68, n. 6, p. 394-424, Nov. 2018.

BRIGELIUS-FLOHE, R. Tissue-specific functions of individual glutathione peroxidases. **Free Radical Biology & Medicine,** v. 27, n. 9-10, p. 951-965, Nov. 1999.

BRIGELIUS-FLOHE, R. et al. Phospholipid-hydroperoxide glutathione peroxidase. Genomic DNA, cDNA, and deduced amino acid sequence. **Journal of Biological Chemistry**, v. 269, n. 10, p. 7342-7348, Mar. 1994.

BRIGELIUS-FLOHE, R. Selenium compounds and selenoproteins in cancer. **Chemistry & Biodiversity**, v. 5, n. 3, p. 389-395, Mar. 2008.

BRIGELIUS-FLOHE, R.; KIPP, A. Glutathione peroxidases in different stages of carcinogenesis. **Biochimica et Biophysica Acta**, v. 1790, n. 11, p. 1555-1568, Nov. 2009.

BRIGELIUS-FLOHE, R.; KIPP, A. Selenium in the redox regulation of the Nrf2 and the Wnt pathway. In: **Methods in Enzymology**, Elsevier, v. 527, chapter 4, p. 65-86, 2013.

BRIGELIUS-FLOHE, R.; MAIORINO, M. Glutathione peroxidases. **Biochimica et Biophysica Acta**, v.1830, n. 5, p. 3289-3303, May. 2013.

BRINKMAN, M. et al. Are men with low selenium levels at increased risk of prostate cancer? **European Journal of Cancer**, v. 42, n. 15, p. 2463-2471, Oct. 2006.

BRODERICK, P. et al. A genome-wide association study shows that common alleles of SMAD7 influence colorectal cancer risk. **Nature Genetics,** v. 39, n. 11, p. 1315-1317, Nov. 2007.

BROWNIE, S. Why are elderly individuals at risk of nutritional deficiency? **International Journal of Nursing Practice**, v. 12, n. 2, p. 110-118, Mar. 2006.

BROWNSON, R. C.; CHANG, J. C.; DAVIS, J. R. Gender and histologic type variations in smoking-related risk of lung cancer. **Epidemiology,** v. 3, n. 1, p. 61-64, Jan. 1992.

BURK, R. F.; et al. Deletion of selenoprotein P upregulates urinary selenium excretion and depresses whole-body selenium content. **Biochimica et Biophysica Acta,** v. 1760, n. 12, p. 1789–1793, Dec. 2006.

BURK, R. F.; HILL, K. E.; MOTLEY, A. K. Plasma selenium in specific and non-specific forms. **Biofactors,** v. 14, n. 1-4, p. 107-114, 2001.

BURK, R. F.; HILL, K. E.; MOTLEY, A. K. Selenoprotein metabolism and function: evidence for more than one function for Selenoprotein P. **The Journal of Nutrition**, v. 133, n. 5, p. 1517S-1520S, May 2003.

BURK, R. F.; HILL, K. E. Selenoprotein P: an extracellular protein with unique physical characteristics and a role in selenium homeostasis. **Annual Review of Nutrition,** v. 25, p. 215–235, Aug. 2005.

BURK, R. F.; HILL, K. E. Selenoprotein P – expression, functions, and roles in mammals. **Biochimica et Biophysica Acta**, v. 1790, n. 11, p. 1441-1447, Nov. 2009.

BURK, R. F.; HILL, K. E. Regulation of selenium metabolism and transport. **Annual Review of Nutrition**, v. 35, p. 109-134, Jul. 2015.

BUTLER, J. A. et al. Selenium distribution in blood fractions of New Zealand women taking organic or inorganic selenium. **American Journal of Clinical Nutrition,** v. 53, n. 3, p. 748-754, Mar. 1991.

CAI, X. et al. Selenium exposure and cancer risk: an updated meta-analysis and meta-regression. **Scientific Reports**, v. 6, n. 19213, Jan. 2016.

CALVO, A. et al. Alterations in gene expression profiles during prostate cancer progression: functional correlations to tumorigenicity and down-regulation of selenoprotein-P in mouse and human tumors. **Cancer Research,** v. 62, n. 18, p. 5325-5335, Sep. 2002.

CAMPA, A. et al. Mortality risk in selenium-deficient HIV-positive children. **Journal of Acquired Immune Deficiency Syndromes and Human Retrovirology**, v. 20, n. 5, p. 508-513, Apr. 1999.

CARDOSO, B. R. et al. Brazil nuts: nutritional composition, health benefits and safety aspects. **Food Research International**, v. 100, pt 2, p. 9-18, Oct. 2017.

CARLSON, B. A. et al. Role of selenium-containing proteins in T-cell and macrophage function. **The Proceedings of the Nutrition Society**, v. 69, n. 3, p. 300-310, Aug. 2010.

CARTER, C. L.; ALLEN, C.; HENSON, D. E. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. **Cancer,** v. 63, n. 1, p. 181-187, Jan. 1989.

CHANG, J. C. et al. Selenium content of Brazil nuts from two geographic locations in Brazil. **Chemosphere,** v. 30, n. 4, p. 801-802, Feb. 1995.

CHEN, Y. C. et al. Dietary selenium supplementation modifies breast tumor growth and metastasis. **International Journal of Cancer,** v. 133, n. 9, p. 2054-2064, Nov. 2013.CHEN, Y. C.; PRABHU, K. S.; MASTRO, A. M. Is selenium a potential treatment for cancer metastasis? **Nutrients,** v. 5, n. 4, p. 1149–1168, Apr. 2013.

CHENG, W. H. et al. Cellular glutathione peroxidase is the mediator of body selenium to protect against paraquat lethality in transgenic mice. **The Journal of Nutrition**, v. 128, n. 7, p. 1070-1076, Jul. 1998.

CLARK, L. C. et al. Effects of selenium supplementation for cancer prevention in patients with carcinoma of the skin: A randomized controlled trial. Nutritional Prevention of Cancer Study Group. **JAMA,** v. 276, n. 24, p. 1957-1963, Dec. 1996.

CLARK, L. C. et al. Plasma selenium concentration predicts the prevalence of colorectal adenomatous polyps. **Cancer Epidemiology, Biomarkers & Prevention,** v. 2, n. 1, p. 41-46, Jan./Feb. 1993.

CLARK, L. C.; et al. Plasma selenium and skin neoplasms: a case control study. **Nutrition and Cancer,** v. 6, n. 1, p. 13-21, 1984.

CLARK, L. C. et al. Decreased incidence of prostate cancer with selenium supplementation: results of a double-blind cancer prevention trial. **British Journal of Urology**, v. 81, n. 5, p. 730-734, May 1998.

CLARK, L. C.; JACOBS, E. T. Environmental selenium and cancer: risk or protection? **Cancer Epidemiology Biomarkers & Prevention,** v. 7, n. 10, p. 847-848, Oct. 1998.

COMBS, G. F. Jr. Biomarkers of selenium status. **Nutrients,** v. 7, n. 4, p. 2209-2236, Mar. 2015.

COMBS JR., G.F. 2005. “Importance of selenium in human nutrition”. In: Proceedings Twenty Years of Selenium Fertilization (Merja Eurola ed.), Helsinki, Finland. **Agrifood Research Reports**, v.69, p.60-70.

COMBS, G. F. JR.; COMBS, S. B. **The role of selenium in nutrition.** New York: Academic Press, 1986.

COMINETTI, C. et al. Associations between glutathione peroxidase-1 pro198leu polymorphism, selenium status, and DNA damage levels in obese women after consumption of brazil nuts. **Nutrition,** v. 27, n. 9, p. 891-896, Sep. 2011. doi:10.1016/j.nut.2010.09.003.

COMINETTI, C.et al. Brazilian nut consumption improves selenium status and glutathione peroxidase activity and reduces atherogenic risk in obese women. **Nutrition Research,** v. 32, n. 6, p. 403-407, Jun. 2012.

COUSSENS, L. M.; WERB, Z. Inflammation and cancer. **Nature,** v. 420, n. 6917, p. 860-867, Dec. 2002.

DAVIS, C. D.; FINLEY, J. W. Chemical versus food forms of selenium in cancer prevention. In: **Functional Foods and Nutraceuticals in Cancer Prevention**, p. 55-85, 2003.

DAVIS, C. D.; UTHUS, E. O. Dietary folate and selenium affect dimethylhydrazine-induced aberrant crypt formation, global DNA methylation and one-carbon metabolism in rats. **Journal of Nutrition**, v. 133, n. 9, p. 2907-2914, Sep. 2003.

DAVIS, C. D.; UTHUS, E. O.; FINLEY, J. W. Dietary selenium and arsenic affect DNA methylation in vitro in Caco-2 cells and in vivo in rat liver and colon. **The Journal of Nutrition**, v. 130, n. 12, p. 2903-2909, Dec. 2000.

DEXTER, D. L. et al. Heterogeneity of tumor cells from a single mouse mammary tumor. **Cancer Research,** v. 38, n. 10, p. 3174-3181, Oct. 1978.

DKHIL, M. A. et al. Selenium nanoparticles attenuate oxidative stress and testicular damage in streptozotocin-induced diabetic rats. **Molecules**, v. 21, n. 11, p. 1517-1529, Nov. 2016.

DOLL, R. The lessons of life: keynote address to the nutrition and cancer conference. **Cancer Research,** v. 52, n. 7 Suppl., p. 2024S-2029S, Apr. 1992.

DOLL, R.; PETO, R. The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today. **Journal of the National Cancer Institute,** v. 66, n. 6, p. 1191-1308, Jun. 1981.

DONALDSON, M. S. Nutrition and cancer: a review of the evidence for an anticancer diet. **Nutrition Journal,** v. 3, n. 19 p. 1-21, Oct. 2004.

DONG, Y. et al. Characterization of the biological activity of gamma-glutamyl-Se-methylselenocysteine: A novel, naturally occurring anticancer agent from garlic. **Cancer Research,** v. 61, n. 7, p. 2923-2928, Apr. 2001.

DONG, Y. et al. Identification of molecular targets associated with selenium-induced growth inhibition in human breast cells using cDNA microarrays. **Cancer Research,** v. 62, n. 3, p. 708-714, Feb. 2002.

DONG, Y. et al. Delineation of the molecular basis for selenium-induced growth arrest in human prostate cancer cells by oligonucleotide array. **Cancer Research,** v. 63, n. 1, p. 52-59, Jan. 2003.

DUMONT, E. et al. Speciation of Se in *Bertholletia excelsa* (Brazil nut): a hard nut to crack? **Food Chemistry,** v. 95, n. 4, p. 684-692, Apr. 2006.

DUMONT, E.; VANHAECKE, F.; CORNELIS, R. Selenium speciation from food source to metabolites: a critical review. **Analytical and Bioanalytical Chemistry**, v. 385, n. 7, p. 1304-1323, Aug. 2006.

DUNN, P. K.; TAYLOR, P. R. Prostate cancer prevention and the selenium and vitamin E cancer prevention trial (SELECT): a selenium perspective. In: **Selenium: Its molecular biology and role in human health,** 3rd ed, New York: Springer, 2012, pp 297-312.

DUNTAS, L. H.; BENVENGA, S. Selenium: an element for life. **Endocrine**, v. 48, n. 3, p. 756-775, Apr. 2015.

ECKERS, J. C. et al. Selenoprotein P Inhibits Radiation-Induced Late Reactive Oxygen Species Accumulation and Normal Cell Injury. International **Journal of Radiation Oncology,** v. 87, n. 3, p. 619-625, Nov. 2013.

EKOUE, D. N. et al. Selenium levels in human breast carcinoma tissue are associated with a common polymorphism in the gene for SELENOP (Selenoprotein P). **Journal of Trace Elements in Medicine and Biology**, v. 39, p. 227-233, Jan. 2017.

ESAKI, N. et al. Enzymatic synthesis of selenocysteine in rat liver. **Biochemistry,** v. 20, n. 15, p. 4492-4496, Jul. 1981.

ESAKI, N. et al. Selenocysteine lyase, a novel enzyme that specifically acts on selenocysteine. **The Journal of Biological Chemistry,** v. 257, n. 8, p. 4386-4391, Apr. 1982.

ETMINAN, M. et al. Intake of selenium in the prevention of prostate cancer: a systematic review and meta-analysis. **Cancer Causes & Control**, v. 16, n. 9, p. 1125-1131, Nov. 2005.

FAGHFURI, E. et al. Dose-response relationship study of selenium nanoparticles as an immunostimulatory agent in cancer-bearing mice. **Archives of Medical Research**, v. 46, n. 1, p. 31-37, Jan. 2015.

FAIRWEATHER-TAIT, S. J. Bioavailability of selenium. **European Journal of Clinical Nutrition,** v. 51, n. Suppl. 1, S20-S23, 1997.

FERGUSON, L. R. et al. Selenium and it's role in the maintenance of genomic stability. **Mutation Research**, v. 733, n. 1-2, p. 100-110, May. 2012.

FERLAY, J. et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. **International Journal of Cancer,** v. 136, n. 5, p. E359-386, Mar. 2015. doi:10.1002/ijc.29210.

FERNANDES, A. P.; GANDIN, V. Selenium compounds as therapeutic agents in cancer. **Biochimica et Biophysica Acta**, v. 1850, n. 8, p. 1642-1660, Aug. 2015.

FERREIRA, K. S. et al. Concentrações de selênio em alimentos consumidos no Brasil. **Revista Panamericana de Salud Pública,** v. 11, n. 3, p. 172–177, 2002.

FLORES-MATEO, G. et al. Selenium and coronary heart disease: a meta-analysis. **The American Journal of Clinical Nutrition**, v. 84, n. 4, p. 762-773, Oct. 2006.

FORDYCE, F. Selenium geochemistry and health. **Ambio,** v. 36, n. 1, p. 94-97, Feb. 2007.

FRANKE, K. W.; PAINTER, E. P. A study of the toxicity and selenium content of seleniferous diets, with statistical consideration. **Cereal Chemistry**, v. 15, p. 1-24, 1938.

FREITAS, S. et al. Meta-análise do teor de selênio em castanha-do-Brasil. **Brazilian Journal of Food Technology,** v. 11, n. 1, p. 54-62, Jan./Mar. 2008.

GABBIANELLI, R. et al. Cypermethrin-induced plasma membrane perturbation on erythrocytes from rats: reduction of fluidity in the hydrophobic core and in glutathione peroxidase activity. **Toxicology,** v. 175, n. 1-3, p. 91-101, Jun. 2002.

GAMMELGAARD, B. et al. Estimating intestinal absorption of inorganic and organic selenium compounds by in vitro flux and biotransformation studies in Caco-2 cells and ICP-MS detection. **Biological Trace Element Research,** v. 145, n. 2, p. 248-256, Feb. 2012.

GANTHER, H. E. 1986. Pathways of Se metabolism including respiratory excretory products. **International Journal of Toxicology,** v. 5, n. 1, p. 1-5, Jan. 1986.

GAO, S. et al. Selenium level and cognitive function in rural elderly Chinese. **American Journal of Epidemiology**, v. 165, n. 8, p. 955-965, Apr. 2007.

GIBSON, R. S. Assessment of trace element status in humans. **Progress in Food and Nutrition Science,** v. 13, n. 2, p. 67-111, 1989.

GILL, J. K. et al. Association of selenium, tocopherols, carotenoids, retinol, and 15-isoprostane F2t in serum or urine with prostate cancer risk: the multiethnic cohort. **Cancer Causes & Control**, v. 20, n. 7, p. 1161-1171, Sep. 2009.

GLATTRE, E.; NYGÅRD, J. F.; AASETH, J. Selenium and cancer prevention: observations and complexity. **Journal of Trace Elements in Medicine and Biology,** v. 26, n. 2-3, p. 168–169, Jun. 2012.

GLATTRE, E. et al. Prediagnostic serum selenium in a case-control study of thyroid cancer. **International Journal of Epidemiology**, v. 18, n. 1, p. 45-49, Mar. 1989.

GOH, J. et al. Mitochondrial targeted catalase suppresses invasive breast cancer in mice. **BMC Cancer**, v. 11, n. 1, p. 191-202, May 2011.

GREENWALD, P. et al. Micronutrients in cancer chemoprevention. **Cancer and Metastasis Reviews,** v. 21, n. 3-4, p. 217–230, 2002.

GUO, C-H.; HSIA, S.; CHEN, P-C. Distribution of selenium and oxidative stress in breast tumor-bearing mice. **Nutrients**, v. 5, n. 2, p. 594-607, Feb. 2013.

HANAHAN, D.; WEINBERG, R. A. The hallmarks of cancer. **Cell**, Elsevier Science, v. 100, n. 1, p. 57–70, 2000.

HARDELL, L. et al. Levels of selenium in plasma and glutathione peroxidase in erythrocytes and the risk of breast cancer. **Biological Trace Element Research**, v. 36, n. 2, p. 99-108, Feb. 1993.

HARRIS, H. R.; BERGKVIST, L.; WOLK, A. Selenium intake and breast cancer mortality in a cohort of Swedish women. **Breast Cancer Research and Treatment**, v. 134, n. 3, p. 1269-1277, Aug. 2012.

HATFIELD, D. L. et al. **Selenium:** Its molecular biology and role in human health. 3rd ed. New York: Springer, 2012.

HERIGSTAD, R. R.; WHITEHAIR, C. K.; OLSON, O. E. Inorganic and organic selenium toxicosis in young swine: comparison of pathologic changes with those in swine with vitamin E-selenium deficiency. **American Journal of Veterinary Research**, v. 34, n. 10, p. 1227-1238, Oct. 1973.

HIDIROGLOU, M. Trace element deficiencies and fertility in ruminants: a review. **Journal of Dairy Science**, v. 62, n. 8, p. 1195-1206, Aug. 1979.

HILL, K. E. et al. Deletion of selenoprotein P alters distribution of selenium in the mouse. **The Journal Biological Chemistry,** v. 278, n. 16, p. 13640-13646, Apr. 2003.

HILL, K. E. et al. Neurological dysfunction occurs in mice with targeted deletion of the selenoprotein P gene. **The Journal of Nutrition,** v. 134, n. 1, p. 157-161, Jan. 2004.

HOFFMANN, F. W. et al. Dietary selenium modulates activation and differentiation of CD4+ T cells in mice through a mechanism involving cellular free thiols. **The Journal of Nutrition**, v. 140, n. 6, p. 1155-1161, Jun. 2010.

HOFFMANN, P. R. Mechanisms by which selenium influences immune responses. **Archivum Immunologiae et Therapiae Experimentalis**, v. 55, n. 5, p. 289-297, Sep-Oct. 2007.

HOLST, B.; WILLIAMSON, G. Nutrients and phytochemicals: from bioavailability to bioefficacy beyond antioxidants. **Current Opinion in Biotechnology**, v. 19, n. 2, p. 73-82, Apr. 2008.

HORVATH, P. M; IP, C. Synergistic effect of vitamin E and selenium in the chemoprevention of mammary carcinogenesis in rats. **Cancer Research,** v. 43, n. 11, p. 5334-5341, Nov. 1983.

HOSNEDLOVA, B. et al. A summary of new findings on the biological effects of selenium in selected animal species – a critical review. **International Journal of Molecular Sciences**, v. 18, n. 10, p. 2209-2255, Oct. 2017.

HU, Y. J.; DIAMOND, A. M. Role of glutathione peroxidase 1 in breast cancer: loss of heterozygosity and allelic differences in the response to selenium. **Cancer Research,** v. 63, n. 12, p. 3347-3351, Jun. 2003.

HUNT, J. R. Bioavailability of iron, zinc, and other trace minerals from vegetarian diets. **The American Journal of Clinical Nutrition**, v. 78, n. 3, p. 633S-639S, Sep. 2003.

HUNTER, D. J. et al. A prospective study of selenium status and breast cancer risk. **JAMA: The Journal of the American Medical Association**, v. 264, n. 9, p. 1128-1131, Sep. 1990.

IMAI, H. et al. Early embryonic lethality caused by targeted disruption of the mouse PHGPx gene. **Biochemical and Biophysical Research Communications,** v. 305, n. 2, p. 278-286, May 2003.

INSTITUTE OF MEDICINE. Food and nutrition board. In: **Dietary References Intakes for Vitamin C, Vitamin E, Selenium and Carotenoids.** Washington: National Academy Press, 2000.

INSTITUTO NACIONAL DE CÂNCER JOSÉ ALENCAR GOMES DA SILVA. Coordenação de PREVENÇÃO E VIGILÂNCIA. Estimativa 2018: incidência de câncer no Brasil. Rio de Janeiro: INCA, 2017. Disponível em: https://www.inca.gov.br/publicacoes/livros/estimativa-2018-incidencia-de-cancer-no-brasil. Acesso em: 26/janeiro/2019.

IP, C. et al. Chemical speciation influences comparative activity of selenium-enriched garlic and yeast in mammary cancer prevention. **Journal of Agricultural and Food Chemistry,** v. 48, n. 6, p. 2062-2070, Jun. 2000.

IP, C.; DONG, Y.; GANTHER, H. E. New concepts in selenium chemoprevention. **Cancer Metastatis Reviews,** v. 21, n. 3-4, p. 281-289, 2002.

IP, C.; LISK, D. J. Modulation of phase I and phase II xenobiotic-metabolizing enzymes by selenium-enriched garlic in rats. **Nutrition and Cancer**, v. 28, n. 2, p. 184-188, 1997.

IP, C.; MEDINA, D. Current concepts of selenium and mammary tumorigenesis. In: **Cellular and Molecular Biology of Mammary Cancer**, Springer, Boston, MA, p. 479-494, 1987.

IP, C.; WHITE, G. Mammary cancer chemoprevention by inorganic and organic selenium: single agent treatment in combination with vitamin E and their effects on in vitro immune functions. **Carcinogenesis,** v. 8, n. 12, p. 1763-1766, Dec. 1987.

JAEGER, E. et al. Common genetic variants at the CRAC1 (HMPS) locus on chromosome 15q13.3 influence colorectal cancer risk. **Nature Genetics,** v. 40, n. 1, p. 26-28, Jan. 2008.

JIANG, C. et al. Selenium-induced inhibition of angiogenesis in mammary cancer at chemopreventive levels of intake. **Molecular Carcinogenesis**, v. 26, n.4, p. 213-225, Dec. 1999.

KANG, D. H. Oxidative stress, DNA damage, and breast cancer. **AACN Clinical Issues,** v. 13, n. 4, p. 540-549, 2002.

KARUNASINGHE, N. et al. DNA stability and serum selenium levels in a high-risk group for prostate cancer. **Cancer Epidemiology, Biomarkers and Prevention**, v. 13, n. 3, p. 391-397, Mar. 2004.

KASAIKINA, M. V.; HATFIELD, D. L.; GLADYSHEV, V. N. Understanding selenoprotein function and regulation through the use of rodent models. **Biochimica et Biophysica Acta**, v. 1823, n. 9, p. 1633-1642, Sep. 2012.

KIPP, A. et al. Four selenoproteins, protein biosynthesis, and Wnt signalling are particularly sensitive to limited selenium intake in mouse colon. **Molecular Nutrition & Food Research,** v. 53, n. 12, p. 1561-1572, Dec. 2009.

KIPP, A. P. et al. The selenoproteins Gpx2, Trxr2 and Trxr3 are regulated by Wnt signaling in the intestinal epithelium. **Biochimica et Biophysica Acta,** v. 1820, n. 10, p. 1588-1596, Oct. 2012.

KIPP, A. P. et al. Revised reference values for selenium intake. **Journal of Trace Elements in Medicine and Biology,** v. 32, p. 195-199, Oct. 2015.

KLEIN, E. A. et al. Select: the selenium and vitamin E cancer prevention trial: rationale and design. Prostate Cancer and Prostatic Diseases, v. 3, n. 3, p. 145-151, Nov. 2000.

KÖHRL, J. et al. Selenium in biology: facts and medical perspectives". **Biological Chemistry,** v. 381, n. 9-10, p. 849-864, Sep. 2000.

KOWALSKA, E. et al. Increased rates of chromosome breakage in BRCA1 carriers are normalized by oral selenium supplementation. **Cancer Epidemiology, Biomarkers and Prevention**, v. 14, n. 5, p. 1302-1306, May 2005.

KRYUKOV, G. V. et al. Characterization of mammalian selenoproteomes. **Science**, v. 300, n. 5624, p. 1439-1443, May 2003.

KUMAR, B. S.; PRIYADARSINI, K. I. Selenium nutrition: how important is it? **Biomedicine & Preventive Nutrition**, v. 4, n. 2, p. 333-341, Apr-Jun. 2014.

LABUNSKYY, V. M.; HATFIELD, D. L.; GLADYSHEV, V. N. Selenoproteins: molecular pathways and physiological roles. **Physiological Reviews**, v. 94, n. 3, p. 739-777, Jul. 2014.

LANKOFF, A.; BANASIK, A.; NOWAK, M. Protective effect of melatonin against nodularin-induced oxidative stress. **Archives of Toxicology,** v. 76, n. 3, p. 158-165, Apr. 2002.

LEE, E. H. et al. Effects of selenium supplements on cancer prevention: meta-analysis of randomized controlled trials. **Nutrition and Cancer,** v. 63, n. 8, p. 1185-1195, Nov. 2011.

LEI, X. G.; CHENG, W-H.; MCCLUNG, J. P. Metabolic regulation and function of glutathione peroxidase-1. **Annual Review of Nutrition**, v. 27, p. 41-61, 2007.

LEVANDER, O. A.; AGER, A. L. JR.; BECK, M. A. Vitamin E and selenium: contrasting and interacting nutritional determinants of host resistance to parasitic and viral infections. **The Proceedings of the Nutrition Society,** v. 54, n. 2, p. 475-487, Jul. 1995.LI, H. et al. A prospective study of plasma selenium levels and prostate cancer risk. **Journal of the National Cancer Institute,** v. 96, n. 9, p. 696–703, May 2004.

LI, H. et al. A prospective study of plasma selenium levels and prostate cancer risk. **Journal of the National Cancer Institute,** v. 96, n. 9, p. 696-703, May 2004.

LIPPMAN, S. M. et al. Effect of selenium and vitamin E on risk of prostate cancer and other cancers: the Selenium and Vitamin E Cancer Prevention Trial (SELECT). **JAMA,** v. 301, n. 1, p. 39-51, Jan. 2009.

LOH, K. et al. Reactive oxygen species enhance insulin sensitivity. **Cell metabolism**, v. 10, n. 4, p. 260-272, Oct. 2009.

MAHABIR, S. et al. Dietary zinc, copper and selenium, and risk of lung cancer. **International Journal of Cancer**, v. 120, n. 5, p. 1108-1115, Mar. 2006.

MANIS, J.; SCHACHTER, D. Active transport of iron by intestine: mucosa iron pools. **American Journal of Physiology,** v. 207, n. 4, p. 893-900. 1964.

MCCLAIN, C. **UA to test selenium to keep disease at bay.** 2002. Disponível em: <http://archive.tobacco.org/news/100214.html>. Acesso em: 15 out. 2018.

MCCONNELL, K. P.; ROTH, D. M.. Respiratory excretion of selenium. **Proceedings of the Society for Experimental Biology and Medicine,** v. 123, n. 3, p. 919-921, Dec. 1966.

MEDINA, D.; MORRISON, D. G. Current ideas on selenium as a chemopreventive agent. **Pathology and Immunopathology Research**, v. 7, n. 3, p. 187-199, 1988.

MEHDI, Y. et al. Selenium in the environment, metabolism and involvement in body functions. **Molecules**, v. 18, n. 3, p. 3292-3311, Mar. 2013.

MISRA, S. et al. Redox-active selenium compounds – from toxicity and cell death to cancer treatment. **Nutrients**, v. 7, n. 5, p. 3536-3556, May. 2015.

MOXON, A. L.; RHIAN, M. Selenium poisoning. **Physiological Reviews**, v. 23, n. 4, p. 305-337, Oct. 1943.

NATIONAL CANCER INSTITUTE. **NCI Chemoprevention Study of Selenium for Non-Small Cell Lung Cancer**. 2005. Disponível em: <http://www.cancer.gov/clinicaltrials/ft-ECOG-5597>. Acesso em: 15 out. 2018.

NAVAS-ACIEN, A.; BLEYS, J.; GUALLAR, E. Selenium intake and cardiovascular risk: what is new? **Current Opinion in Lipidology**, v. 19, n. 1, p. 43-49, Feb. 2008.

NEGRO, R. et al. The influence of selenium supplementation on postpartum thyroid status in pregnant women with thyroid peroxidase autoantibodies. **The Journal of Clinical Endocrinology & Metabolism**, v. 92, n. 4, p. 1263-1268, Apr. 2007.

OHSHIMA, H.; BARTSCH, H. Chronic infections and inflammatory processes as cancer risk factors: possible role of nitric oxide in carcinogenesis. **Mutation Research,** v. 305, n. 2, p. 253-264, Mar. 1994.

OLIVIERI, O. et al. Low selenium status in the elderly influences thyroid hormones. **Clinical Science**, v. 89, n. 6, p. 637-642, Dec. 1995.

Organização Mundial da Saúde (OMS). Estimated cancer incidence, mortality and prevalence worldwide [acesso em 31 out 2018]. Disponível em: http://www.who.int/news-room/fact-sheets/detail/cancer. 2018.

PACHECO, A. M., SCUSSEL, V. M. Selenium and aflatoxin levels in raw Brazil nuts from the Amazon basin. **Journal of Agricultural and Food Chemistry,** v. 55, n. 26, p. 11087-11092, Dec. 2007.

PAPP, L.V. et al. From selenium to selenoproteins: synthesis, identity, and their role in human health. **Antioxidants & Redox Signaling,** v. 9, n. 7, p. 775-806, Jul. 2007.

PAREKH, P. P. et al. Concentrations of selenium, barium, and radium in Brazil nuts. **Journal of Food Composition and Analysis,** v 21, n. 4, p. 332-335, Jun. 2008.

PELUCCHI, C. et al. Cancer risk associated with alcohol and tobacco use: focus on upper aero-digestive tract and liver. **Alcohol Research & Health,** v. 29, n. 3, p. 193-198, 2006.

PETERS, U.; TAKATA, Y. Selenium and the prevention of prostate and colorectal cancer. **Molecular Nutrition & Food Research**, v. 52, n. 11, p. 1261-1272, Nov. 2008.

PIETINEN, P.; VARTIAINEN, E.; SEPPANEN, R.; ARO, A.; PUSKA, P. Changes in diet in Finland from 1972 to 1992: impact on coronary heart disease risk. **Preventive Medicine**, v. 25, n. 3, p. 243-250, May 1996.

PULASKI, B. A.; OSTRAND-ROSENBERG, S. Reduction of established spontaneous mammary carcinoma metastases following immunotherapy with major histocompatibility complex class II and B7.1 cell-based tumor vaccines. **Cancer Research**, v. 58, n. 7, p. 1486-1493, Apr. 1998.

PULASKI, B. A.; OSTRAND-ROSENBERG, S. Mouse 4T1 breast tumor model. **Current Protocols in Immunology**, v. 39, n. 1, p. 20.2.1-20.2.16, May 2001.

RAJPATHAK, S. et al. Toenail selenium and cardiovascular disease in men with diabetes. **Journal of the American College of Nutrition**, v. 24, n. 4, p. 250-256, Aug. 2005.

RAY, A. L. et al. Low serum selenium and total carotenoids predict mortality among older women living in the community: the women's health and aging studies. **The Journal of Nutrition**, v. 136, n. 1, p. 172-176, Jan. 2006.

RAYMAN, M. P. The importance of selenium to human health. **Lancet,** v. 356, n. 9225, p. 233-241, Jul. 2000.

RAYMAN, M. P. Selenium in cancer prevention: a review of the evidence and mechanism of action. **Proceedings of the Nutrition Society,** v. 64, n. 4, p. 527-542, Nov. 2005.

RAYMAN, M. P. Food-chain selenium and human health: emphasis on intake. **British Journal of Nutrition**, v. 100, n. 2, p. 254-268, Aug. 2008.

RAYMAN, M. P. Selenoproteins and human health: insights from epidemiological data. **Biochimica et Biophysica Acta**, v. 1790, n. 11, p. 1533-1540, Nov. 2009.

RAYMAN, M. P. Selenium. In: CONNOR, J. R.; LEE, S. Y. **Bioactive Compounds and Cancer**. Milner, J. A.; Romagnolo, D. F., eds. Humana Press/Springer, p. 411-448. 2010.

RAYMAN, M. P. Selenium and human health. **Lancet,** v. 379, n. 9822, p. 1256-1268, Mar. 2012.

REJALI, L.; JAAFAR, M. H.; ISMAIL, N. H. Serum selenium level and other risk factors for breast cancer among patients in a Malaysian hospital. **Environmental Health and Preventive Medicine**, v. 12, n. 3, p. 105-110, May 2007.

RESZKA, E. Selenoproteins in bladder cancer. **Clinica Chimica Acta**, v. 413, n. 9-10, p. 847-854, May 2012.

RIESE, C. et al. Selenium-dependent pre- and posttranscriptional mechanisms are responsible for sexual dimorphic expression of selenoproteins in murine tissues. **Endocrinology**, v. 147, n. 12, p. 5883-5892, Dec. 2006.

RITCHIE, M D. et al. Multifactor-dimensionality reduction reveals high-order interactions among estrogen-metabolism genes in sporadic breast cancer. **American Journal of Human Genetics,** v. 69, n. 1, p. 138-147, Jul. 2001. doi: 10.1086/321276.

ROMAN, M.; JITARU, P. BARBANTE, C. Selenium Biochemistry and Its Role for Human Health. **Metallomics,** v. 6, n. 1, p. 25-54, Jan. 2014.

ROS, E.; MATAIX, J. Fatty acid composition of nuts - Implications for cardiovascular health. **The British Journal of Nutrition,** v. 96 n. Suppl. 2, p. S29-35, Nov. 2006.

SALONEN, J. T. et al. Association between cardiovascular death and myocardial infarction and serum selenium in a matched-pair longitudinal study. **The Lancet**, v. 320, n. 8291, p. 175-179, Jul. 1982.

SANDER, C. S. et al. Role of oxidative stress and the antioxidant network in cutaneous carcinogenesis. **International Journal of Dermatology**, v. 43, n. 5, p. 326-335, May 2004.

SANDSTROM, B. Micronutrient interactions: effects on absorption and bioavailability. **British Journal of Nutrition**, v. 85, n. S2, p. S181-S185, May. 2001.

SANMARTIN, C. et al. Selenium compounds, apoptosis and other types of cell death: an overview for cancer therapy. **International Journal of Molecular Sciences**, v. 13, n. 8, p. 9649-9672, Aug. 2012.

SAPPEY, C. et al. Stimulation of glutathione peroxidase activity decreases HIV type 1 activation after oxidative stress. **AIDS Research and Human Retroviruses**, v. 10, n. 11, p. 1451-1461. 1994.

SATO, F., et al. Genomic tumor evolution of breast cancer. **Breast Cancer**, v. 23, n. 1, p.4-11, 2016.

SCHOMBURG, L.; SCHWEIZER, U.; KOHRLE, J. Selenium and selenoproteins in mammals: extraordinary, essential, enigmatic. **Cellular and Molecular Life Science,** v. 61, n. 16, p.1988-1995, Aug. 2004.

SCHRAUZER, G. N. Selenomethionine: A review of its nutritional significance, metabolism and toxicity. **The Journal of Nutrition,** v. 130, n. 7, p. 1653-1656, Jul. 2000.

SCHRAUZER, G. N. The nutritional significance, metabolism and toxicology of selenomethionine. **Advances in Food and Nutrition Research,** v. 47, p. 73-112 2003.

SCHRAUZER, G. N.; RHEAD, W. J. Interpretation of the methylene blue reduction test of human plasma and the possible cancer protecting effect of selenium. **Experientia,** v. 27, n. 9, p. 1069-1071, Sep. 1971.

SCHWARZ, K.; FOLTZ, C. M. Selenium as an integral part of factor 3 against dietary liver degeneration. **Journal of American Chemical Society,** v. 79, n. 12, p. 3292-3293, 1957.

SCHWEIZER, U. et al. Selenium and brain function: a poorly recognized liaison. **Brain Research Reviews**, v. 45, n. 3, p. 164-178, Jul. 2004.

SEO, Y. R.; KELLEY, M. R.; SMITH, M. Selenomethionine regulation of p53 by a ref1-dependent redox mechanism. **Proceedings of the National Academy of Science of the United States of America,** v. 99, n. 22, p. 14548-14553, Oct. 2002.

SHAHAR, S. et al. Antioxidant intake and status, and oxidative stress in relation to breast cancer risk: A case-control study. **Asian Pacific Journal of Cancer Prevention,** v. 9, n. 2, p. 343-350, Apr./Jun. 2008.

SHAHAR, A. et al. Plasma selenium is positively related to performance in neurological tasks assessing coordination and motor speed. **Movement Disorders**, v. 25, n. 12, p. 1909-1915, Sep. 2010.

SHAMBERGER, R. J.; FROST, D. V. Possible protective effect of selenium against human cancer. **Canadian Medical Association Journal,** v. 100, n. 14, p. 682, Apr. 1969.

SHAMBERGER, R. J.; WILLIS, C. E. Selenium distribution and human cancer mortality. **CRC Critical Reviews in Clinical Laboratory Sciences,** v. 2, n. 2, p. 211-221, Jun. 1971.

SHIOBARA, Y.; SUZUKI, K. T.. Binding of selenium (administered as selenite) to albumin after efflux from red blood cells. **Journal of Chromatography,** v. 710, n. 1-2, p. 49-56, Jun. 1998.

SILVA JUNIOR, E. C. et al. Natural variation of selenium in Brazil nuts and soils from the Amazon region. **Chemosphere,** v. 188, p. 650-658, Dec. 2017.

SINGLETARY, S. E. et al. Revision of the American Joint Committee on Cancer staging system for breast cancer. **Journal of Clinical Oncology**, v. 20, n. 17, p. 3628-3636, Sep. 2002.

SMITH, M. L. et al. Selenium compounds regulate p53 by common and distinctive mechanisms. **Anticancer Research**, v. 24, n. 3A, p. 1401-1408, May-Jun. 2004.

SOLOVYEV, N. et al. Selenium, selenoprotein P, and Alzheimer's disease: is there a link? **Free Radical Biology and Medicine**, v. 127, p. 124-133, Nov. 2018.

SOTGIA, F.; MARTINEZ-OUTSCHOORN, U. E.; LISANTI, M. P. Mitochondrial oxidative stress drives tumor progression and metastasis: should we use antioxidants as a key component of cancer treatment and prevention? **BMC Medicine,** v. 9, 2011.

STEINBRENNER, H.; SIES, H. Protection against reactive oxygen species by selenoproteins. **Biochimica et Biophysica Acta**, v. 1790, n. 11, p. 1478-1485, Nov. 2009.

STEINBRENNER, H.; SPECKMANN, B.; KLOTZ, L-O. Selenoproteins: antioxidant selenoenzymes and beyond. **Archives of Biochemistry and Biophysics**, v. 595, p. 113-119, Apr. 2016.

STEINNES, E. Soils and geomedicine. **Environmental Geochemistry and Health,** v. 31, n. 5, p. 523-535, Oct. 2009.

STOCKLER-PINTO, M. B. et al. Effect of Brazil nut supplementation on the blood levels of selenium and glutathione peroxidase in hemodialysis patients. **Nutrition**, v. 26, n. 11-12, p. 1065-1069, Nov. 2010.

STOCKLER-PINTO, M. B. et al. Brazil nut (Bertholletia excelsa, H.B.K.) improves oxidative stress and inflammation biomarkers in hemodialysis patients. **Biological Trace Element Research,** v. 158, n. 1, p. 105-112, Apr. 2014.

STOCKLER-PINTO, M. B. et al. Effect of selenium supplementation via Brazil Nut (Bertholletia Excelsa, Hbk) on thyroid hormones levels in hemodialysis patients: a pilot study. **Nutricion Hospitalaria,** v. 32, n. 2, p. 1808-1812, Oct. 2015.

SUNDE, R. A. Regulation of glutathione peroxidase-1 expression. In: **Selenium: Its Molecular Biology and Role in Human Health**, Springer, NY, p. 149-160, 2006.

SUNDE, R. A. et al. Selenium status highly regulates selenoprotein mRNA levels for only a subset of the selenoproteins in the selenoproteome. **Bioscience Reports,** v. 29, n. 5, p. 329-338, Jun. 2009.

SURAI, P. F. Effect of the selenium and vitamin E content of the maternal diet on the antioxidant system of the yolk and the developing chick. **British Poultry Science,** v. 41, n. 2, p. 235-243, May 2000.

SURAI, P. F. et al. Effect of vitamin E and selenium of cockerel diets on glutathione peroxidase activity and lipid peroxidation susceptibility in sperm, testes and liver. **Biological Trace Element Research,** v. 64, n. 1-3, p. 119-132, 1998.

SURAI, P. F. **Natural antioxidants in avian nutrition and reproduction.** Nottingham: Nottingham University Press, 2002.

SURAI, P. F. **Selenium in Nutrition and Health.** Nottingham: Nottingham University Press, 2006.

SURAI, P. F. Selenium-vitamin E interactions: Does 1+1 equal more than 2?. In: Nutritional biotechnology in the feed and food industries. In: **Proceedings of Alltech's 19th Annual Symposium**. Nottingham University Press, Nottingham, UK, p. 59-76, 2003.

SUZUKI, K. T. Metabolomics of selenium: Se metabolites based on speciation studies. **Journal of Health Science,** v. 51, n. 2, p. 107-114, 2005.

SUZUKI, K. T.; OGRA, Y. Metabolic pathway for selenium in the body: speciation by HPLC-ICP-MS with enriched Se. **Food Additives and Contaminants,** v. 19, n. 10, p. 974-983, Oct. 2002.

SUZUKI, Y.; SAKAI, T.; FURUTA, N. Isolation of Selenoprotein-P and Determination of Se Concentration Incorporated in Proteins in Human and Mouse Plasma by Tandem Heparin Affinity and Size-Exclusion Column HPLC-ICPMS. **Analytical Sciences,** v. 28, n. 3, p. 221-224, 2012.

TAKEMOTO, A. S.; BERRY, M. J.; BELLINGER, F. P. Role of selenoprotein P in Alzheimer's disease. **Ethnicity & Disease**, v. 20, n. 1, Suppl. 1, p. S1-92-95, Winter 2010.

THIRUNAVUKKARASU, C.; SAKTHISEKARAN, D. Effect of dietary selenite on N-nitrosodiethylamine-induced and phenobarbital promoted multistage hepatocarcinogenesis in rat: reflection in some minerals. **Biomedicine and Pharmacotherapy,** v. 57, n. 9, p. 416-421, Nov. 2003.

THOMSON, C. D. Selenium speciation in human body fluids. **The Analyst,** v. 123, n. 5, p. 827-831, May 1998.

THOMSON, C. D. Assessment of requirements for selenium and adequacy of selenium status: A review. **European Journal of Clinical Nutrition,** v. 58, n. 3, p. 391-402, Mar. 2004.

THOMSON, C. D. et al. Brazil nuts: an effective way to improve selenium status. **The American Journal of Clinical Nutrition**, v. 87, n. 2, p. 379-384, Feb. 2008.

TOBE, R. et al. Differences in redox regulatory systems in human lung and liver tumors suggest different avenues for therapy. **Cancers**, v. 7, n. 4, p. 2262-2276, Nov. 2015.

TORRE, L. A. et al. Global Cancer Incidence and Mortality Rates and Trends--An Update. **Cancer Epidemiology, Biomarkers & Prevention**, v. 25, n. 1, p. 16-27, Jan. 2016. doi:10.1158/1055-9965.EPI-15-0578.

TOUAT-HAMICI, Z. et al. Selective up-regulation of human selenoproteins in response to oxidative stress. **The Journal of Biological Chemistry**, v. 289, n. 21, p. 14750-14761, May 2014.

TSUJI, P. A. et al. Dietary selenium levels affect selenoprotein expression and support the interferon-gama and IL-6 immune response pathways in mice. **Nutrients**, v. 7, n. 8, p. 6529-6549, Aug. 2015.

ULLREY, D. E. Biochemical and physiological indicators of selenium status in animals. **Journal of Animal Science,** v. 65, n. 6, p. 1712-1726, Dec. 1987.

UNDERWOOD, E. J. Trace elements in human and animal nutrition. 4th Edn. New York: **Academic Press**, p. 303-345, 1977.

UNNI, E. et al. Se-methylselenocysteine activates caspase-3 in mouse mammary epithelial tumor cells in vitro. **BioFactors**, v. 14, n. 1-4, p. 169-177, Jan. 2001.

UNNI, E. et al. Se-methylselenocysteine inhibits phosphatidylinositol 3-kinase activity of mouse mammary epithelial tumor cells in vitro. **Breast Cancer Research**, v. 7, n. 5, p. R699-R707, Jul. 2005.

URSINI, F. The world of glutathione peroxidases". **Journal of Trace Elements in Medicine and Biology,** v. 14, p. 116, 2000.

URSINI, F.; MAIORINO, M.; ROVERI, A. Phospholipid hydroperoxide glutathione peroxidase (PHGPx): More than an antioxidant enzyme? **Biomedical and Environmental Sciences,** v. 10, n. 2-3, p. 327-332, Sep. 1997.

VALENTINE, W. M. et al. Brainstem axonal degeneration in mice with deletion of selenoprotein P. **Toxicologic Pathology,** v. 33, n. 5, p. 570–576, 2005.

VLEMINCKX, K., et al. Genetic manipulation of E-cadherin expression by epithelial tumor cells reveals an invasion suppressor role. **Cell**, v.66, p.107–119, 1991.
VONDERHEIDE, A. P. et al. Characterization of selenium species in Brazil nuts by HPLC-ICP-MS and ES-MS. **Journal of Agricultural and Food Chemistry,** v. 50, n. 20, p. 5722-5728, Sep. 2002

WALLENBERG, M. et al. Selenium induces a multi-targeted cell death process in addition to ROS formation. **Journal of Cellular and Molecular Medicine**, v.18, n. 4, p. 671-684, Apr. 2014.

WALLENBERG, M.; MISRA, S.; BJORNSTEDT, M. Selenium cytotoxicity in cancer. **Basic & Clinical Pharmacology & Toxicology**, v. 114, n. 5, p. 377-386, Feb. 2014.

WANG, Y. et al. Common 5p15.33 and 6p21.33 variants influence lung cancer risk. **Nature Genetics,** v. 40, n. 12, p. 1407-1409, Dec. 2008.

WATERS, D. J. et al. Prostate cancer risk and DNA damage: translational significance of selenium supplementation in a canine model. **Carcinogenesis**, v. 26, n. 7, p. 1256-1262, Jul. 2005.

WEIGELT, B.; PETERSE, J. L.; VAN'T VEER, L. J. Breast cancer metastasis: markers and models. **Nature Rev. Cancer**, v.5, p.591–602, 2005.

WHANGER, P. D. Selenoprotein W: a review. **Cellular and Molecular Life Sciences**, v. 57, n. 13-14, p. 1846-1852, Dec. 2000.

WHANGER, P. et al. Metabolism of subtoxic levels of selenium in animals and humans. **Annals of Clinical and Laboratory Science,** v. 26, n. 2, p. 99-113, Mar./Apr. 1996.

WILLETT, W. C. et al. Prediagnostic serum selenium and risk of cancer. **The Lancet,** v. 322, n. 8342, p. 130-134, Jul. 1983.

WOLFFRAM, S. Absorption and metabolism of selenium: difference between inorganic and organic sources. In: 15th Alltech's Annual Symposium. **Biotechnology in the Feed Industry**. Nottingham: Nottingham University Press, pp. 547-566, 1999.

WOLFFRAM, S.; BERGER, B.; GRENACHER, B; SCHARRER, E. Transport of selenoamino acids and their sulfur analogues across the intestinal brush border membrane of pigs. **The Journal of Nutrition**, v. 119, n. 5, p. 706-712, May 1989.

WROBEL, J. K.; POWER, R.; TOBOREK, M. Biological activity of selenium: revisited. **International Union of Biochemistry and Molecular Biology Life**, v. 68, n. 2, p. 97-105, Feb. 2016.

XIA, Y. et al. Effectiveness of selenium supplements in a low-selenium area of China. **The American Journal of Clinical Nutrition,** v. 81, n. 4, p. 829-834, Apr. 2005.

XIA, Y. et al. Optimization of selenoprotein P and other plasma selenium biomarkers for the assessment of the selenium nutritional requirement: a placebo-controlled, double-blind study of selenomethionine supplementation in selenium-deficient Chinese subjects. **The American Journal of Clinical Nutrition,** v. 92, n. 3, p. 525-531, Sep. 2010.

YANG, J. Brazil nuts and associated health benefits: a review. **LWT - Food Science and Technology,** v. 42, n. 10, p. 1573-1580, Dec. 2009.

YOSHIZAWA, K. et al. Study of prediagnostic selenium level in toenails and the risk of advanced prostate cancer. **Journal of the National Cancer Institute**, v. 90, n. 16, p. 1219-1224, Aug. 1998.

YU, M-W. et al. Plasma selenium levels and risk of hepatocellular carcinoma among men with chronic hepatitis virus infection. **American Journal of Epidemiology**, v. 150, n. 4, p. 367-374, Aug. 1999.

ZAIDI, S. M. K. R.; BANU, N. Antioxidant potential of vitamins A, E and C in modulating oxidative stress in rat brain. **Clinica Chimica Acta**, v. 340, n. 1-2, p. 229-233, Feb. 2004.

ZHANG, Y. et al. Comparative analysis of selenocysteine machinery and selenoproteome gene expression in mouse brain identifies neurons as key functional sites of selenium in mammals. **Journal of Biological Chemistry**, v. 283, n. 4, p. 2427-2438, Jan. 2008.

ZHUO, H.; SMITH, A. H.; STEINMAUS, C. Selenium and lung cancer: a quantitative analysis of heterogeneity in the current epidemiological literature. **Cancer Epidemiology Biomarkers & Prevention**, v. 13, n. 5, p. 771-778, May 2004.

ZORZATO, F. et al. Functional effects of mutations identified in patients with multiminicore disease. **IUBMB Life**, v. 59, n. 1, p. 14-20, Jan. 2007.

## SEGUNDA PARTE – ARTIGO

# ARTIGO 1 – INITIAL INHIBITION OF 4T1 MAMMARY TUMOR GROWTH IN MICE FED WITH BRAZILIAN NUTS SELENIUM-RICH SUPPLEMENTED DIET

**(VERSÃO PRELIMINAR)**

**Artigo preparado de acordo com as normas para submissão do periódico Nutrients**

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*Article*

# Initial Inhibition of 4T1 Mammary Tumor Growth in Mice Fed with Brazilian nuts Selenium-rich Supplemented Diet

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**Abstract:** We investigated the effects of dietary selenium (Se) supplementation on 4T1 mammary tumor growth, blood total Se concentration and hepatic glutathione peroxidase (GPx) activity in *Balb/c* mice. Initially, 30 female mice received subcutaneous inoculation of 4T1 cells. After 5 days, all animals showed palpable tumors and they were randomly assigned into three groups: a control group (Se-adequate) receiving diet containing adequate level of total Se (0.15 ppm), and two supplemented groups with diet containing 1.4 ppm of total Se derived either from Brazilian nuts (Se-Nuts) or from selenomethionine (SeMet). Animals received diets for 28 days, starting six days after tumor induction. Tumors were measured every 48 hours in each mouse to estimate tumor volume through 28 days. At the end of the experiment, animals were euthanized and blood and hepatic samples were collected. Data was submitted to ANOVA followed by post hoc Tukey's HSD test. Both Se-supplemented diets reduced tumor growth until the eighth day of feeding when compared to the Se-adequate group ($p < 0.05$). SeMet group presented average Se blood concentration of 0.507 µg/mL, significantly higher ($p < 0.05$) than the Se-adequate group (0.342 µg/mL), but with no difference from Se-Nuts group (0.461 µg/mL), which presented an intermediate value. Average hepatic GPx enzyme activities in the Se-adequate, SeMet and Se-Nuts groups were, respectively, 6663.583, 7486.480 and 7862.332 nmo/min/mL, and no significant difference was observed between them. There was no correlation between Se blood concentration, hepatic GPx activity and tumor growth. It was concluded that dietary Se supplementation (by both SeMet or Brazilian nuts) reduced tumor growth during the initial stages. Additional studies are encouraged in order to elucidate mechanisms promoted by Se intake in the different carcinogenic stages.

**Keywords:** *Bertholletia*; neoplasms; selenomethionine; selenoprotein P; glutathione peroxidase; antioxidants; nutritional sciences; enzymology; tumor burden; dietary supplements.

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## 1. Introduction

Cancer is a leading cause of death in countries of all income levels [1]. Breast cancer - the most prevalent type in women - represented almost 12% of all cancer cases considering both genders (1.7 million people) and 6% of all deaths (522,000 people) in the world [2]. Although breast cancer is generally recognised to be a multifactorial disease [3], oxidative stress has been related to a higher risk of cancer development and to a faster neoplasic progression [4, 5, 6, 7, 102].

The imbalance between antioxidant systems and the production of reactive oxygen species (ROS), such as superoxide ($O_2^-$) and hydrogen peroxide ($H_2O_2$), characterizes the oxidative stress [8], which can damage DNA [9-11] and lead to mutations in tumor-related genes [12, 13]. Therefore, the

concurrent use of antioxidants to control ROS formation has been proposed, although the evidence remains uncertain [14].

Selenium is an essential micronutrient [15, 16] known to play an important role in the antioxidant body system [17, 18]. Although with some controversial epidemiological data [19, 20], some evidence suggest that inorganic and organic forms of selenium negatively affect cancer initiation and progression [21-26]. These protective effects, which includes even less mortality in cancer patients [20], have been associated to different mechanisms [27], specially the selenoprotein glutathione peroxidase (GPx) [28, 20].

The GPxs are a family of antioxidant enzymes [29, 30] recognized for having protective effects against tumor development [31, 32]. By evaluating the genotype of women with and without breast cancer, Hu & Diamond (2003) [33] reported loss of heterozygosity within the gene for glutathione peroxidase 1 (GPx-1), implicating this enzyme as a key role in breast cancer development. The main function of this family is to neutralize hydrogen peroxide and organic hydroperoxides in the intracellular and extracellular compartments [34]. Since enzymatic activity of some GPxs is directly proportional to selenium intake, there is a strong link between dietary selenium deficiency and increase in oxidative stress [35, 30].

Dietary Se can be presented in the organic form, such as selenocysteine (SeCys) and selenomethionine (SeMet), or inorganic forms, such as selenite ($SeO(OH)_2$) and selenate ($SeO_2(OH)_2$) [36]. Natural sources include cereals, nuts, meat, some vegetables and sea food. The amount of selenium in food is very variable and can be related to the selenium content in the soil, beyond other factors [37, 38]. Brazilian nuts (*Bertholletia excelsa*) are known of having great amounts of selenium, varying from 0.2 to 512 μg [37, 39, 40], with substantial bioavailability [41-43].

Dietary Se supplementation is associated with reduced incidence of breast cancer [44]. However, the efficacy of Se supplementation, depends on the dose and chemical form of Se [19, 20]. Distinct forms of Se in various concentrations can express dramatically different biological effects [9, 45]. Chen et al (2013) [46] reported that SeMet supplementation protected against 4T1 primary tumor growth, whereas selenite supplementation allowed higher number of metastasis in comparison to a control group. Although scientific reports have shown encouraging results with Se as a therapeutic agent [47], we hypothesize this response may depend on which stage of carcinogenesis the consumption begins [48, 49]. Detecting the optimum form and the ideal amount of Se supplementation is a potential role for ROS-mediated mechanisms in cancer therapeutics [50, 51] expecting that someday Se-compounds might be used to provide inhibition of tumor growth with remarkable specifity [52]. Therefore, this study aimed to evaluate the effects of Se dietary supplementation (SeMet and Brazilian nuts) on tumor growth, blood Se level and GPx activity in a 4T1 breast cancer model.

## 2. Materials and Methods

### *2.1. Experimental Animals*

Seven-week-old female *Balb/c* mice (*Mus musculus*) weighting 20 to 24 g were provided by Central Animal Laboratory (UFLA) in Lavras, MG, Brazil, and distributed into 6 boxes with 410 x 340 x 175 mm dimensions with 5 mice each. The room had constant temperature of 25 ± 2ºC and 12 hours light-dark cycles.

Previously to the experiment, animals were kept under an adaptation period of seven days receiving deionized water and commercial feeding. Mice had food and water *ad libitum* and their weight was determined periodically. The animal study was approved by UFLA's Ethical Comittee in Animal Use (CEUA/UFLA) under protocol number 079/16.

### *2.2. 4T1 cells*

The Comparative Pathology Laboratory (LPC), Pathology Department of Federal University of Minas Gerais (UFMG), provided the 4T1 cells. The 4T1 cell line was obtained from the American Type Culture Collection (ATCC, USA) and was routinely cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 1% penicillin/streptomycin and 10% fetal bovine serum (FBS; Thermo Fisher Scientific), at 37$^{o}$C, in a humidified 5% $CO_2$ atmosphere. Cultures were tested for mycoplasma contamination by immunofluorescence staining with 1 µg/mL solution of 4',6-diamino-2-phenilindole (DAPI; Sigma-Aldrich).

### *2.3. Experimental Design*

On the inoculation day, each animal received 0.1 mL subcutaneous injection containing 1 x $10^6$ 4T1 cells diluted in phosphate buffered saline (PBS; Sigma). The solution was injected into mice's flank and tumors were measured daily until all animals presented palpable tumors.

All animals had detectable tumors at the 5$^{th}$ day after inoculation, when tumor started to be measured every 48 hours. On the sixth day, animals were randomly distributed into one of three experimental diets, as shown in Figure 1.

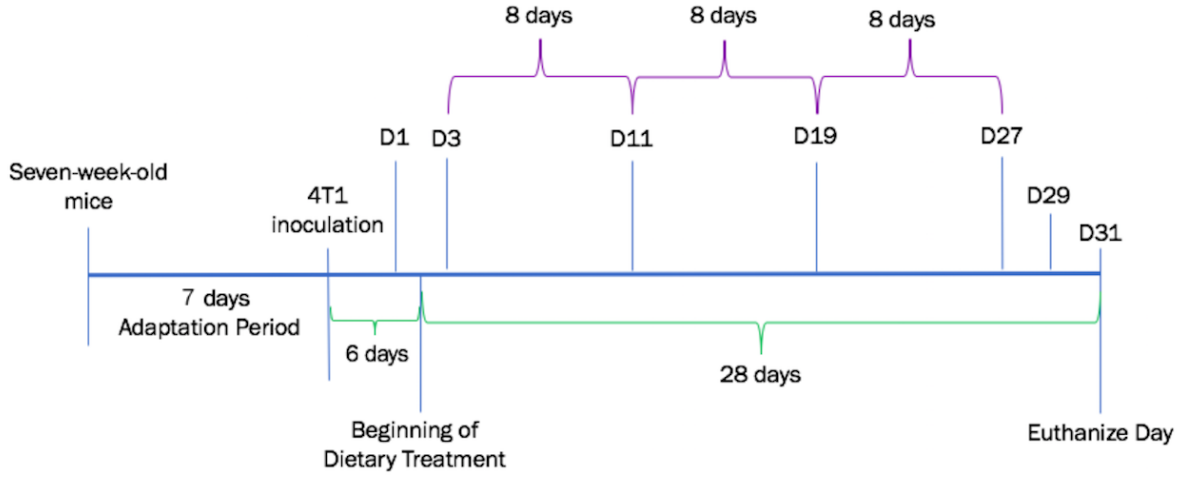


**Figure 1.** Fluxogram representing Experimental design along time. Thirty female *Balb/c* mice were maintained under adaptation period for 7 days before tumor cells inoculation. Five days after cancer induction (all animals showed palpable tumors), we started estimating tumor volume (D1). Tumor volume was recorded every 48 hours and tumor growth was compared between groups along 8 days intervals (from D3 to D11, D11 to D19, and D19 to D27). Total tumor growth comprised tumor volume difference between D29 and D1. Animals started experimental diets six days after 4T1 inoculation to characterize a treatment study. After 28 feeding days, at D31, all animals were euthanized for blood and hepatic tissue collection.

In order to avoid unnecessary pain, animals showing respiratory and/or behavior alterations along the Experimental were euthanized before the study endpoint.

### *2.4. Measurement of Tumor Growth*

Tumor diameters were measured using a pachymeter (Western, measurement 0.05 mm, ref. 1944). Tumor volume was estimated according to previous studies [53, 46] using the formula: Tumor volume = (length X width$^2$)/2, where "length" is the longer diameter and "width" is the shorter diameter. Tumor growth was calculated based on four measurements through a total of 28 days, as described further.

The first tumor measurement occurred on D1, which was five days after 4T1 cells inoculation and one day before initiating experimental diets. Afterwards, tumor volume was recorded every 48 hours.

Periodical tumor growth between groups was compared using records obtained at days D3, D11, D19 and D27. Also, these four dates provided an estimate for total tumor growth through 3 periods of 8 days. Tumoral growth was also evaluated using regression models using volumes obtained every 48 hours interval from D1 to D29.

Total tumor growth during 28 days (from D1 to D29) was also compared among groups. Euthanize was conducted after 28 complete days under dietary treatment.

*2.5. Experimental Diets*

Mice received experimental diets during 28 days, with a Se-adequate (0.15 ppm total Se) or a Se-supplemented (1.4 ppm total Se) levels. Adequate level was based on AIN-93 M ration for laboratory rodents and supplementary levels were based on previous reports [104, 105, 46, 106]. Total Se amount in the diets was analytically determined by atomic absorption spectrophotometer with graphite furnace (GF-AAS) [37]. Se-adequate diet contained sodium selenate while Se-supplemented diets contained either SeMet (Selisseo 2%, Adisseo®, Brazil), or Brazilian nuts (*Bertholletia excelsa*) provided by Aruanã Farm (Itacoatira, AM, Brazil) (Table 1).

**Table 1.** Ingredients of experimental diets.

| | **Se-adequate (0.15 ppm Se)** | **Se-supplemented (1.4 ppm Se)** | |
|---|---|---|---|
| | Sodium selenate | SeMet | Se-Nuts |
| **Starch (g)** | 609.8 | 609.8 | 609.8 |
| **Casein (g)** | 200 | 200 | 197.5 |
| **Cellulose (g)** | 50 | 50 | 49.2 |
| **Sucrose (g)** | 50 | 50 | 48 |
| **Soybean oil (mL)** | 40 | 40 | 30.5 |
| **AIN-93 M Mineral Mix*[1] (g)** | 35 | 35 | 35 |
| **AIN-93 M Vitamin Mix*[2] (g)** | 10 | 10 | 10 |
| **Methionine (g)** | 3 | 3 | 3 |
| **Choline (g)** | 2 | 2 | 2 |
| **BHT (g)** | 0.2 | 0.2 | 0.2 |
| **Selisseo® 2% Se (g)** | - | 0.0035 | - |
| **Brazilian nut (g)** | - | - | 14.8 |
| **kcal/kg diet** | 3759.2 | 3759.2 | 3753.8 |

Thirty female *Balb/c* mice were fed *ad libitum* with one of the three diets for 28 days. Experimental groups involved diets containing 0.15 ppm total Se (Se-adequate) in the form of sodium selenate and also two supplemented groups containing 1.4 ppm Se (SeMet and Se-Nuts).

*[1] Mix by Rhoster® Co. Mineral element content (g/kg mix): 357 g of calcium carbonate, anhydrous (40.04% Ca); 250 g of potassium phosphate, monobasic (22.76% P; 28.73% K); 209.806 g of powdered sucrose; 74 g of sodium chloride (39.34% Na; 60.66% Cl); 46.6 g of potassium sulfate (44.87% K; 18.39% S); 28 g of potassium citrate, tri-potassium, monohydrate (36.16% K); 24 g of magnesium oxide (60.32% Mg); 6.06 g of ferric citrate (16.5% Fe); 1.65 g of zinc carbonate (52.14% Zn); 1.45 g of sodium meta-silicate, 9 hydrate (9.88% Si); 0.63 g of manganous carbonate (47.79% Mn); 0.30 g of cupric carbonate (57.47% Cu); 0.275 g of chromium potassium sulfate, 12 hydrate (10.42% Cr); 0.0815 g of boric acid (17.5% B); 0.0635 g of sodium fluoride (45.24% F); 0.0318 g of nickel carbonate (45% Ni); 0.0174 g of lithium chloride (16.38% Li); 0.01025 g of sodium selenate, anhydrous (41.79% Se); 0.01 g of potassium iodate (59.3% I); 0.00795 g of ammonium paramolybdate, 4 hydrate (4.34% Mo); 0.0066 g of ammonium vanadate (43.55% V).

*[2] Mix by Rhoster® Co. Vitamin content (g/kg mix): 974.655 g of powdered sucrose; 15 g of vitamin E (all-*rac*-a-tocopheryl acetate) (500 IU/g); 3 g of nicotinic acid; 2.5 g of vitamin B-12 (cyanocobalamin) (0.1% in mannitol); 1.6 g of Ca pantothenate; 0.8 g of vitamin A (all-*trans*-retinyl palmitate) (500,000 IU/g); 0.7 g of pyridoxine-HCl; 0.6 g of thiamin-HCl; 0.6 g of riboflavin; 0.25 g of vitamin $D_3$ (cholecalciferol) (400,000 IU/g); 0.2 g of folic acid; 0.075 g of vitamin K (phylloquinone); 0.020 g of D-biotin.

Se content and centesimal composition of Brazilian nuts samples were analyzed in order to calculate the necessary amount of material to reach 1.4 ppm in the diet. Experimental diets were balanced with similar calories and macronutrients proportions. Nuts were evaluated for dry matter, humidity, oil, protein, mineral residues and crude fiber according to AOAC (2005) [54], and were also submitted to α and γ tocopherol quantifications, according to previous report [109].

For Se content analysis of the Brazilian nuts, five small paper bags containing 4 nuts each were dried using an oven at 60ºC until constant weight (~72 h). Then, samples were shelled and grinded with a portable electrical mill (A11 basic Analytical mill, IKA®, Staufen, Germany). The digestion process started with the samples inside glass tubes receiving 6 mL of nitro-perchloric acid at proportion 2:1 (v/v). Extracts were left overnight (~12 h) and, then, the batch was digested.

The digestion procedure, initiated at 50ºC and increased 50ºC every 30 minutes until 200ºC. Analytical determination of total Se in the samples was performed using an atomic absorption spectrophotometer with graphite furnace (GF-AAS). We used for quality control, for each batch, a standard reference material (White Clover - BCR 402, Institute for Reference Materials and Measurements, Geel, Belgium) containing 6.70 mg $kg^{-1}$ of Se was included. The average recovery rate for the SRM (*n*=2) was 78.38%.

Selisseo 2% Se (Adisseo®, Brazil) was added to the SeMet diet as a source of hydroxy-SeMet ($CH_3Se$-$(CH_2)_2$-CH(OH)-COOH). It is a white powder containing 5% of SeMet.

Diets were mixed in special bowels previously cleaned with nitric acid solution 10% ($HNO_3$) and staff manipulated all ingredients with nitrile gloves.

### *2.6. Blood and Hepatic Sampling, Preparation, Storage and Analysis*

After twenty-eight days receiving experimental diets, all 30 animals were anesthetized using intraperitoneal injection of Ketamin® (90 mg/kg) and Thiopental sodium® (60 mg/kg) and euthanized by cervical displacement. All materials were manipulated with nitrile gloves and washed with nitric acid cleaning solution 10% ($HNO_3$).

Blood was collected by intracardiac puncture and immediately stored on ice using heparinized tubes. Samples were analyzed at Chemical Analyzes Laboratory (LACHEM, RS, Brazil) for whole blood total selenium evaluation. Samples were digested through acid method (USEPA 3050B) and submitted to hydride generation atomic absorption spectrometry (HG-AAS), as described by Olson, Palmer e Cary (1975) [55].

Liver samples were rinsed with a PBS solution (pH 7.4), to remove any red blood cells and clots, upon collection. Next, they were immediately stored in liquid nitrogen and then stored at -80ºC ultrafreezer for posterior laboratory analyzes.

*2.7. Measurement of Hepatic GPx-1 Activity*

On the day of analysis, hepatic samples were homogenized in 1 mL of cold buffer (50 mM Tris-HCl, pH 7.5, 5 mM EDTA and 1 mM DTT) per 100 miligrams of tissue with 3 cycles of 10 seconds, at 13,000 rpm speed (T 25 basic Ultra-Turrax, IKA®, Staufen, Germany). Homogenates were then centrifugated at 17.760 x g for 15 minutes at 4ºC to collect the supernatant and assay samples were kept on ice in accordance to the manufacturer protocol (Glutathione Peroxidase Assay Kit, Cayman Chemical Company®, Ann Arbor, USA). Enzymatic activity was measured at every 30 seconds for 6 minutes in microplate reader with absorbance at 340 nm.

Protein concentrations were measured by an adaptation of Bradford assay (1976) [56]. As standard, bovine serum albumin (BSA; Sigma®) was diluted into 10 different concentrations from 0.3 mg/mL to 3.0 mg/mL, while samples were diluted at 1:100. Then, 125 µL of Bradford Reagent (Bio-Rad Protein Assay Dye Reagent Concentrate, Bio-Rad®, Cat #5000006) was added for every 25 µL of sample and incubated at gentle shaking for 5 minutes. Microplates were red with absorbance at 595 nm using spectrometer and Gen5 Software.

*2.8. Statistical Analysis*

Data of total Se blood concentration, hepatic GPx activity and tumor growth were submitted to Analysis of Variance (One way ANOVA) [57] and, when significant, means were compared among treatments using Tukey's HSD test with the package Emmeans v2.23 [58] in R 3.4.4 [59]. Tumor growth along time was analyzed by means of repeated measures ANOVA using volumes recorded at days D3, D11, D19 and D27. Pearson correlation coefficient was calculated for blood Se concentration, GPx activity and tumor growth in 24 days (from D3 to D27).

Tumor volume at every 48 hours for each group was submitted to regression analysis, and the equations were compared by the slope provided for each treatment.

## 3. Results

### *3.1. Se concentration and centesimal composition of Brazilian nuts*

Se mean concentration in Brazilian nuts was 95.403 mg/kg. Centesimal composition of the analyzed Brazilian nuts is shown below in Table 2.

**Table 2.** Centesimal composition of Brazilian nuts from Aruanã Farm (Itacoatira, AM).

| Sample | Dry Matter (%) | Humidity (%) | Fat (%) | Protein (%) | Mineral Residue (%) | Crude Fiber (%) |
|---|---|---|---|---|---|---|
| R1 | 98.69 | 1.31 | 58.68 | 17.60 | 3.44 | 5.27 |
| R2 | 98.78 | 1.22 | 61.03 | 16.39 | 3.04 | 5.04 |
| R3 | 98.80 | 1.20 | 58.70 | 17.44 | 3.55 | 5.43 |
| **Mean** | 98.76 | 1.24 | 59.47 | 17.14 | 3.34 | 5.25 |

Samples of Brazilian nuts were divided into triplicates (R1, R2 and R3) for evaluation of dry matter, humidity, fat, protein, mineral residue and crude fiber.

Values for α and γ tocopherols were expressed in mg tocophero l.100 $g^{-1}$ of nuts oil. Average level for α-tocopherol was 0.038 ± 0.007 mg, while for γ-tocopherol it was 0.320 ± 0.010 mg.

### *3.2. Effects of dietary Se on tumor growth*

All animals had palpable tumors by the $5^{th}$ day after 4T1 inoculation. Diets initiated in the $6^{th}$ day. At the first 8 days after dietary treatment (*i.e.*, from D3 to D11) tumor growth was significantly lower in Se-supplemented groups (SeMet and Se-Nuts) when compared to Se-adequate diet ($p < 0.05$). However, this tumor suppression was not maintained, since after D11 significant difference in tumor growth among groups did not persist (Table 3) and there was no effect of diets on total tumor growth after 28 days (Figure 2).

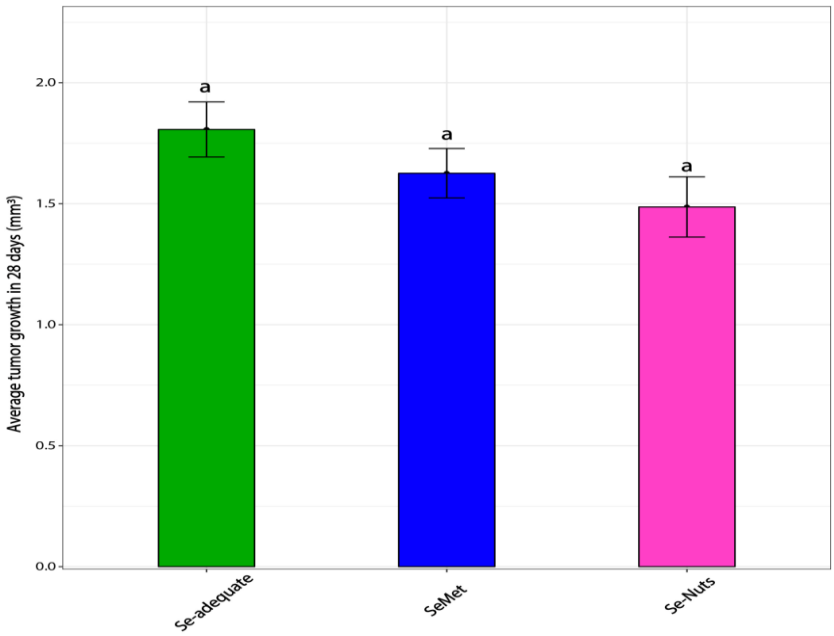


**Figure 2.** Estimated (mean ± EPM) total tumor growth ($mm^3$) through 28 days (from D1 to D29 date) in three different experimental groups: Se-adequate (0.15 ppm total Se) and Se-supplemented diets, which were SeMet or Se-Nuts (1.4 ppm total Se). Tumor volume was calculated based on the formula: Tumor volume = (length X $width^2$)/2, where "length" is the longer diameter and "width" is the shorter diameter. Error bars represent the standard error of the mean ($n$ = 7 for each group). Average values followed by the same letters do not differ statistically by the Tukey test at $p < 0.05$.

**Table 3.** Estimated tumor growth (mean ± SD) of each experimental group divided by 8 days interval between dates D3 to D11, from D11 to D19 and from D19 to D27.

| **Tumor growth ($mm^3$)** | **D3-D11** | **D11-D19** | **D19-D27** |
|---|---|---|---|
| Se-adequate | 0.289 ± 0.0917 [a] | 0.558 ± 0.2540 [a] | 0.685 ± 0.0760 [a] |
| Se-supplem. SeMet | 0.175 ± 0.0494 [b] | 0.411 ± 0.0963 [a] | 0.759 ± 0.1280 [a] |
| Se-supplem. Se-Nuts | 0.151 ± 0.0311 [b] | 0.404 ± 0.0885 [a] | 0.814 ± 0.1970 [a] |

Tumor growth calculated by differences in tumor volume estimated in four dates by measuring two diameters with digital caliper. Tumor volume was estimated using the formula: *Tumor volume = (length X* $width^2$*)/2*, where "length" is the longer diameter and "width" is the shorter diameter. Average values followed by the same letters in the column do not differ statistically by the Tukey test ($p < 0.05$).

In order to compare tumor growth, regression models were performed for tumor volume every 48 hours for each group (Figure 3).

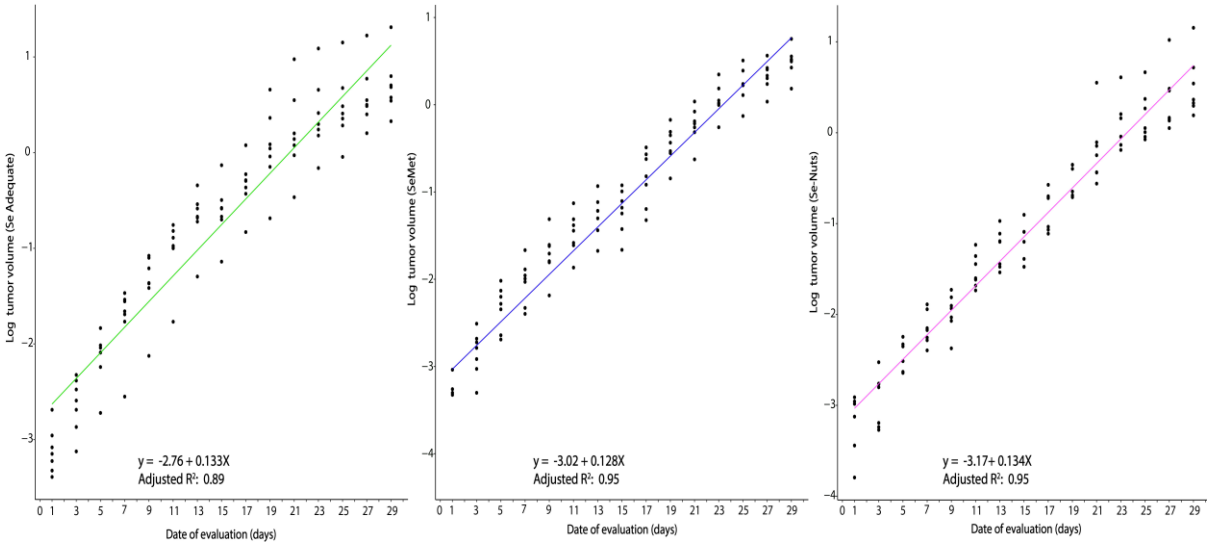


**Figure 3.** Regression model for tumor volume growth along 28 days (from date D1 to D29), every 48 hours, for mice receiving Se-adequate diet (0.15 ppm total Se), SeMet diet (1.4 ppm total Se) and Se-Nuts diet (1.4 ppm total Se). The respective slope values for each group were 0.133 ($R^2$ = 0.89), 0.128 ($R^2$ = 0.95) and 0.134 ($R^2$ = 0.95). Tumor volume was calculated based on the formula: Tumor volume = (length X width$^2$)/2, where "length" is the longer diameter and "width" is the shorter diameter.

### *3.3. Effects of dietary Se on blood Se concentration and hepatic GPx-1 activity*

Significant statistical difference ($p < 0.05$) for blood Se concentration was observed only between Se-adequate (0.15 ppm Se) and SeMet (1.4 ppm Se), as shown in Figure 4A. No significant statistical difference was observed between experimental groups for hepatic GPx-1 activity (Figure 4B).

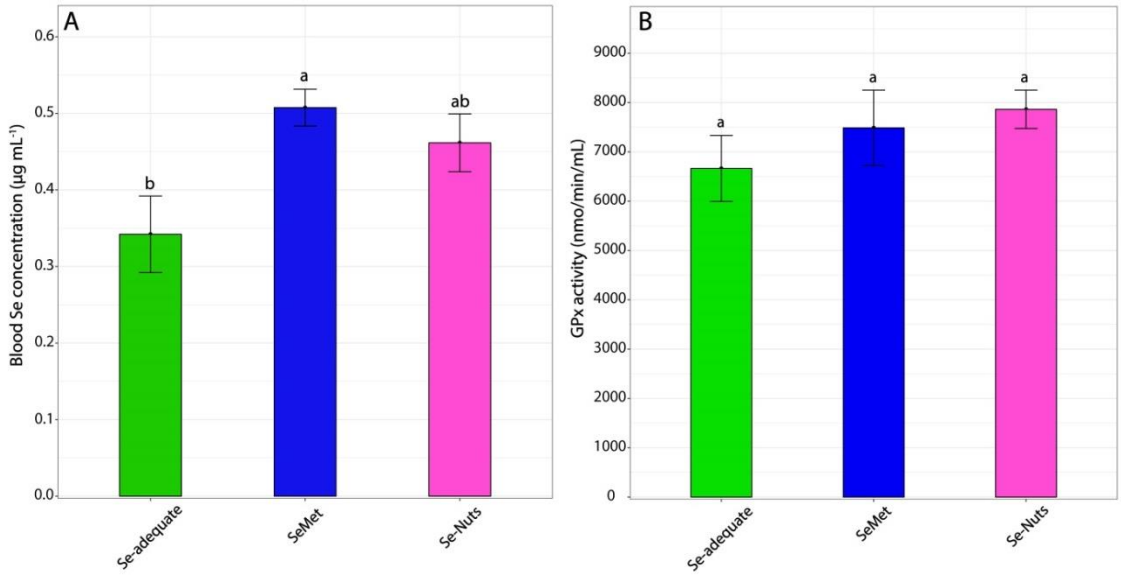


**Figure 4. (**A) Blood Se concentration (µg mL$^{-1}$) and (B) hepatic GPx-1 activity among different dietary groups: Se-adequate (0.15 ppm total Se), SeMet (1.4 ppm total Se) and Se-Nuts (1.4 ppm total Se). Error bars represent the standard error of the mean ($n$ = 7). Average values followed by the same letters do not differ statistically by the Tukey test at $p < 0.05$.

*3.5. Pearson Correlation*

There were no significant correlations between blood Se concentration, GPx activity and tumor growth in 24 days (Figure 5).

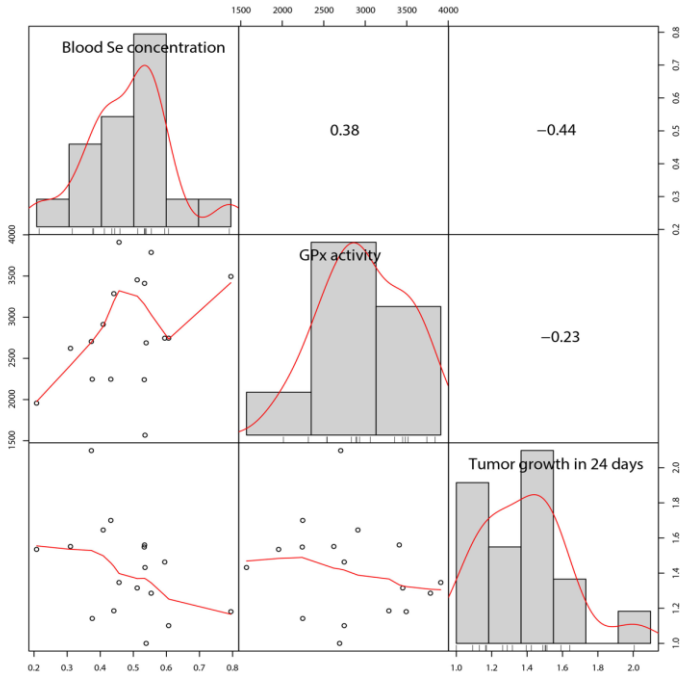


**Figure 5.** Pearson correlation between blood Se concentration, hepatic GPx activity and tumor growth in 24 days ($p < 0.05$).

## 4. Discussion

The present study aimed to investigate whether dietary Se supplementation affected breast cancer growth associated to blood Se concentration and/or hepatic GPx-1 activity. All animals had palpable tumors by the 5$^{th}$ day after 4T1 inoculation, so diets initiated at the 6$^{th}$ day. At the first eight days of dietary treatment, tumor growth was significantly lower in Se-supplemented groups (SeMet and Se-Nuts) when compared to those of control diet (Se-adequate). This brings the hypothesis that, by the end of these first 8 days of Se enriched diet, probably intestinal active transporters for organic Se saturated [60, 18, 61, 62] leading to stabilization in organic Se absorption. This saturation may explain the concept that Se-supplementation may be more effective in individuals with Se deficiency [63, 20, 11], since there was no extra protection after Se supplementation.

Tumor growth inhibition provided by the SeMet in the first 8 days can be due to SelP antioxidant activity in the plasma [64-67], since this group presented the highest blood Se concentration [68]. Other possible mechanisms previously associated with selenium anticancer effects include modulation of p53 tumor-suppressor protein [69], SBP1 [70, 71], plasma GPx activity [72],

Wnt signaling [73], induction of cancer cells apoptosis [74] and inhibition of tumor angiogenesis [75, 76].

The short-term delay on tumor growth observed in the SeMet group contrasts with reports by Chen et al. (2013) [46] which showed, through all 15 days of measurement, significant suppression of primary tumor growth ($p < 0.001$) by SeMet-supplemented diet (3 ppm L-SeMet from Sabinsa Corporation, East Windsor, NJ) when compared to the Se-deficient group (< 0.01 ppm Se). Additionally, on the 16$^{th}$ day after cancer cell inoculation, tumor volume was already affected by the Se status. This may be due to differences in diet regimen. In our study, diets were introduced after tumor inoculation, while Chen et al. initiated diets 3 months previously to 4T1 cells inoculation. Since mice erythrocytes have a 43 days half-life period [77], they might allow a longer period for GPx-1 synthesis in red blood cells [78]. It is interesting that tumor progression was also different between both studies. In the referred article, it took 2 weeks for all mice to develop palpable tumors (in comparison to 5 days in the present study).

Results from another study performed by Song et al. (2015) [70] also contrast with ours, since SeMet supplementation for 28 days was able to reduce total tumor growth compared to PBS. Higher differences were observed only after the 19$^{th}$ day of feeding. Mice of the former Experimental received individual oral administered SeMet (using irrigation needle stomach) while ours Se was included in the feeding. Differences in daily consumption of experimental diets by each animal may be a possible explanation for these conflicting results. Although individual doses are better to determine a dose-effect relationship, using stomach needle daily is a stressful event for long experiments involving nutrients. Additionally, the therapeutic intervention by Song and coworkers occurred at earlier carcinogenesis stage, probably promoting different anticancer effects by involving different selenoproteins [79]. Finally, this comparison highlights possible food matrix influence, which can alter Se bioaccessibility and bioavailability, affecting, therefore, biological effects from dietary intake [80, 81].

The matrix of Brazilian nuts is quite complex and, therefore, it may interfere on the activity of its Se-compounds [82, 83]. It has been reported that Se-species present in Brazilian nuts may include 74% to 96% of SeMet and the rest mostly SeCys [82-85], although it was observed that only SeMet was bioaccessible [86]. The lack of statistical difference of blood Se levels from Se-Nuts animals when compared to those of control group, may have two possible reasons: 1) Se provided by nuts could have been less absorbed than the pure SeMet group (since SeMet is reported to be the most bioavailable Se compound) [87]. 2) Considering that tumor growth was significantly lower in both Se-supplemented groups at the first eight days of dietary therapy, another much more plausible hypothesis suggested is that Se from Brazilian nuts is less retained by the body than the Se from SeMet group [88, 89]. It is known that vitamin E can also be present in Brazilian nuts [90] and its interaction with Se should be taken into consideration [91], since it can enhance antioxidant defense in the organism [SURAI, 2003]. But, since the results for $\alpha$ and $\gamma$ tocopherols in this study would provide only 2.39%

of vitamin E daily nutritional requirement for humans, we hypothesized this amount was negligible, explaining why there was no further increase of protection period against tumor growth in Se-Nuts group.

Brazilian nuts contain high levels of unsaturated fatty acids as both monounsaturated (MUFAs) and polyunsaturated (PUFAs) [92, 93], and changes in the activity of enzymes from GPx family have been described during PUFA supplementation [94, 95]. Consumption of Brazilian nuts by obese women for 8 weeks was reported to increase GPx-1 erythrocyte activity, but the association between GPx activity and erythrocyte Se concentration was not the same among different genotype groups [96]. GPx-1 belongs to a group of stress-related selenoproteins [97] and, since GPx-1 allelic identity is associated to breast cancer development [33], it was expected to observe decreased tumor growth associated with increased hepatic GPx-1 activity in the Se-Nuts group compared to the control.

Tumor-bearing mice exhibited lower GPx activity in plasma in previous studies [5] and the activity of GPx-1 in tissues is more sensitive to dietary Se deficiency than other selenoproteins [98, 99]. Therefore, we expected a difference between Se-adequate and SeMet groups. Although hepatic GPx-1 activity was similar among groups, hepatic GPx-1 activity in the control group (0.15 ppm Se) was similar to those found in a previous study [100].

In summary, organic Se-supplemented diets were effective in suppressing tumor growth in the first 8 days of tumor detection, but this inhibition was not maintained by the end of the experiment, although there was significant difference between blood Se levels of SeMet and Se-adequate groups. Therefore, data presented in this study suggest that both Se-supplemented diets, with 1.4 ppm of SeMet or Brazilian nuts, provided only a short-term delay of tumor growth, which supports the idea that Se-supplementation is more effective in individuals with low Se-status [46, 63, 11]. Additionally, Se supplemented from Brazilian nuts did not improve blood Se concentration as the SeMet diet, although there were no differences in their hepatic GPx-1 activity. This suggests that SeMet supplementation may not affect hepatic GPx-1 before improving blood selenoproteins, which involves mainly plasma SelP, extracellular GPx-3, erythrocytes GPx-1 and lower concentrations of SeMet, trimethylselenonium ion and selenosugar in red blood cells [78, 101].

## 5. Conclusions

Dietary Se-supplementation may provide some delay against breast cancer progression in the first stages when initiated after tumor detection. More research is needed to elucidate the specific mechanisms in which Se-compounds are important in order to develop therapeutic protocols.

Although both Se-supplemented diets (SeMet and Se-Nuts) contained 1.4 ppm of total Se, only the SeMet group showed higher blood Se concentration. Since hepatic GPx-1 activity did not respond to either Se-supplemented diets, evaluation of these parameters (blood Se concentration and

hepatic GPx-1 activity) suggest that SeMet supplementation may not affect hepatic GPx-1 before improving blood selenoproteins.

## 6. Supplementary Materials

### *6.1. List of abbreviations*

Se: Selenium
GPx: Glutathione peroxidase
GPx-1: Glutathione Peroxidase type 1
SeMet: Selenomethionine
SelP: Selenoprotein P
ROS: Reactive oxygen species
SeCys: Selenocysteine
PBS: Phosphate buffered saline
GF-AAS: Atomic absorption spectrophotometer with graphite furnace
α: Alpha
γ: Gamma
HG-AAS: Hydride generation atomic absorption spectrometry

## 7. Declarations

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## 8. References

[1] Torre, L. A., R. L. Siegel, E. M. Ward, and A. Jemal. 2016. "Global Cancer Incidence and Mortality Rates and Trends--An Update." *Cancer Epidemiology Biomarkers & Prevention* 25 (1): 16–27. doi:10.1158/1055-9965.EPI-15-0578.

[2] Ferlay, Jacques, Isabelle Soerjomataram, Rajesh Dikshit, Sultan Eser, Colin Mathers, Marise Rebelo, Donald Maxwell Parkin, David Forman, and Freddie Bray. 2015. "Cancer Incidence and Mortality Worldwide: Sources, Methods and Major Patterns in GLOBOCAN 2012." *International Journal of Cancer* 136 (5). Wiley-Liss Inc.: E359–86. doi:10.1002/ijc.29210.

[3] Ritchie, Marylyn D., Lance W. Hahn, Nady Roodi, L. Renee Bailey, William D. Dupont, Fritz F. Parl, and Jason H. Moore. 2001. "Multifactor-Dimensionality Reduction Reveals High-Order Interactions among Estrogen-Metabolism Genes in Sporadic Breast Cancer." *The American Journal of Human Genetics* 69 (1): 138–47. doi:10.1086/321276.

[4] Cai, Xianlei, Chen Wang, Wanqi Yu, Wenjie Fan, Shan Wang, Ning Shen, Pengcheng Wu, Xiuyang Li, and Fudi Wang. 2016. "Selenium Exposure and Cancer Risk: An Updated Meta-Analysis and Meta-Regression." *Scientific Reports* 6 (January). Nature Publishing Group. doi:10.1038/srep19213.

[5] Guo, Chih Hung, Simon Hsia, and Pei Chung Chen. 2013. "Distribution of Selenium and Oxidative Stress in Breast Tumor-Bearing Mice." *Nutrients* 5 (2). MDPI AG: 594–607. doi:10.3390/nu5020594.

[6] Goh, Jorming, Linda Enns, Soroosh Fatemie, Heather Hopkins, John Morton, Christina Pettan-Brewer, and Warren Ladiges. 2011. "Mitochondrial Targeted Catalase Suppresses Invasive Breast Cancer in Mice." *BMC Cancer* 11 (May). doi:10.1186/1471-2407-11-191.

[7] Sotgia, Federica, Ubaldo E. Martinez-Outschoorn, and Michael P. Lisanti. 2011. "Mitochondrial Oxidative Stress Drives Tumor Progression and Metastasis: Should We Use Antioxidants as a Key Component of Cancer Treatment and Prevention?" *BMC Medicine*. doi:10.1186/1741-7015-9-62.

[8] Nelson, David L., Cox, Michael, M., [tradução: Veiga, Ana B.G., et al.], revisão técnica: Termignoni, Carlos, [et al.]. 2014. "Princípios de bioquímica de Lehninger" *Artmed*, 6. ed., 1298 p., ISBN 978-85-8271-072-2.

[9] Fernandes, Aristi P., and Valentina Gandin. 2015. "Selenium Compounds as Therapeutic Agents in Cancer." *Biochimica et Biophysica Acta - General Subjects*. Elsevier. doi:10.1016/j.bbagen.2014.10.008.

[10] Frederiks, Wilma M., Klazina S. Bosch, Kees A. Hoeben, Jan van Marle, and Sigrun Langbein. 2010. "Renal Cell Carcinoma and Oxidative Stress: The Lack of Peroxisomes." *Acta Histochemica* 112 (4): 364–71. doi:10.1016/j.acthis.2009.03.003.

[11] Rayman, Margaret P. 2005. "Selenium in Cancer Prevention: A Review of the Evidence and Mechanism of Action." *The Proceedings of the Nutrition Society* 64 (4): 527–42. doi:10.1079/PNS2005467.

[12] Bera, Soumen, Viviana De Rosa, Walid Rachidi, and Alan M. Diamond. 2013. "Does a Role for Selenium in DNA Damage Repair Explain Apparent Controversies in Its Use in Chemoprevention?" *Mutagenesis*. doi:10.1093/mutage/ges064.

[13] Klaunig, James E, and Lisa M Kamendulis. 2004. "The Role of Oxidative Stress in Carcinogenesis." *Annual Review of Pharmacology and Toxicology* 44: 239–67. doi:10.1146/annurev.pharmtox.44.101802.121851.

[14] Lawenda, Brian D., Kara M. Kelly, Elena J. Ladas, Stephen M. Sagar, Andrew Vickers, and Jeffrey B. Blumberg. 2008. "Should Supplemental Antioxidant Administration Be Avoided during Chemotherapy and Radiation Therapy?" *Journal of the National Cancer Institute*. doi:10.1093/jnci/djn148.

[15] Duntas, Leonidas H., and Salvatore Benvenga. 2015. "Selenium: An Element for Life." *Endocrine*. Humana Press Inc. doi:10.1007/s12020-014-0477-6.

[16] Papp, Laura Vanda, Jun Lu, Arne Holmgren, and Kum Kum Khanna. 2007. "From Selenium to Selenoproteins: Synthesis, Identity, and Their Role in Human Health." *Antioxidants & Redox Signaling* 9 (7): 775–806. doi:10.1089/ars.2007.1528.

[17] Hasanvand, Amin; Abbaszadeh Abolfazl; Darabi Saeideh; Nazari Afshin; Gholami, Mohammadreza; Kharazmkia, Ali. 2017. "Evaluation of selenium on kidney function following ischemic injury in rats; protective effects and antioxidant activity." *J Renal Inj Prev* 6 (2): 93-98. doi: 10.15171/jrip.2017.18.

[18] Mehdi, Youcef, Jean Luc Hornick, Louis Istasse, and Isabelle Dufrasne. 2013. "Selenium in the Environment, Metabolism and Involvement in Body Functions." *Molecules*. doi:10.3390/molecules18033292.

[19] Chen, Yu-Chi, K Sandeep Prabhu, and Andrea M Mastro. 2013. "Is Selenium a Potential Treatment for Cancer Metastasis?" *Nutrients* 5 (4): 1149–68. doi:10.3390/nu5041149.

[20] Surai, Peter F. 2006. *Selenium in Nutrition and Health*. Edited by Nottingham. *Nottingham University Press*. Nottingham University Press. http://www.ncbi.nlm.nih.gov/pubmed/726511.

[21] Glattre, Eystein, Jan F. Nygård, and Jan Aaseth. 2012. "Selenium and Cancer Prevention: Observations and Complexity." *Journal of Trace Elements in Medicine and Biology* 26 (2–3): 168–69. doi:10.1016/j.jtemb.2012.04.021.

[22] Rayman, Margaret P. 2012. "Selenium and Human Health." *The Lancet*. doi:10.1016/S0140-6736(11)61452-9.

[23] Fordyce, Fiona. 2007. "Selenium Geochemistry and Health." *Ambio* 36 (1): 94–97. doi:10.1579/0044-7447(2007)36[94:SGAH]2.0.CO;2.

[24] Li, Haojie, Meir J. Stampfer, Edward L. Giovannucci, J. Steven Morris, Walter C. Willett, J. Michael Gaziano, and Jing Ma. 2004. "A Prospective Study of Plasma Selenium Levels and Prostate Cancer Risk." *Journal of the National Cancer Institute* 96 (9). Oxford University Press: 696–703. doi:10.1093/jnci/djh125.

[25] Rayman, Margaret P. 2000. "The Importance of Selenium to Human Health." *Lancet*. Elsevier Limited. doi:10.1016/S0140-6736(00)02490-9.

[26] Clark, L.C.; Jacobs, E.T. 1998. "Environmental selenium and cancer: risk or protection?" *Cancer Epidemiology Biomarkers & Prevention*, AACR, v. 7, n. 10, p. 847–848.

[27] Ferguson, Lynnette R., Nishi Karunasinghe, Shuotun Zhu, and Alice H. Wang. 2012. "Selenium and Its' Role in the Maintenance of Genomic Stability." *Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis*. doi:10.1016/j.mrfmmm.2011.12.011.

[28] Hatfield, Dolph L., Petra A. Tsuji, Bradley A. Carlson, and Vadim N. Gladyshev. 2014. "Selenium and Selenocysteine: Roles in Cancer, Health, and Development." *Trends in Biochemical Sciences*. doi:10.1016/j.tibs.2013.12.007.

[29] Brigelius-Flohé, Regina, and Matilde Maiorino. 2013. "Review: Glutathione Peroxidases." *BBA - General Subjects - Biochim. Biophys. Acta* 1830: 3289–3303. doi:10.1016/j.bbagen.2012.11.020.

[30] Brigelius-Flohé, R, K D Aumann, H Blöcker, Gerhard Gross, M Kiess, K D Klöppel, M Maiorino, A Roveri, R Schuckelt, and F Usani. 1994. "Phospholipid-Hydroperoxide Glutathione Peroxidase. Genomic DNA, CDNA, and Deduced Amino Acid Sequence." *The Journal of Biological Chemistry* 269 (10): 7342–48. http://www.ncbi.nlm.nih.gov/pubmed/8125951.

[31] Tobe, Ryuta, Bradley A. Carlson, Petra A. Tsuji, Byeong Jae Lee, Vadim N. Gladyshev, and Dolph L. Hatfield. 2015. "Differences in Redox Regulatory Systems in Human Lung and Liver Tumors Suggest Different Avenues for Therapy." *Cancers* 7 (4). MDPI AG: 2262–76. doi:10.3390/cancers7040889.

[32] Barrett, Caitlyn W., Wei Ning, Xi Chen, Jesse Joshua Smith, Mary K. Washington, Kristina E. Hill, Lori A. Coburn, et al. 2013. "Tumor Suppressor Function of the Plasma Glutathione Peroxidase Gpx3 in Colitis-Associated Carcinoma." *Cancer Research* 73 (3): 1245–55. doi:10.1158/0008-5472.CAN-12-3150.

[33] Hu, Ya Jun, and Alan M. Diamond. 2003. "Role of Glutathione Peroxidase 1 in Breast Cancer: Loss of Heterozygosity and Allelic Differences in the Response to Selenium." *Cancer Research*63 (12): 3347–51.

[34] Lei, Xin Gen, Wen-Hsing Cheng, and James P. McClung. 2007. "Metabolic Regulation and Function of Glutathione Peroxidase-1." *Annual Review of Nutrition* 27 (1): 41–61. doi:10.1146/annurev.nutr.27.061406.093716.

[35] Touat-Hamici, Zahia, Yona Legrain, Anne Laure Bulteau, and Laurent Chavatte. 2014. "Selective Up-Regulation of Human Selenoproteins in Response to Oxidative Stress." *Journal of Biological Chemistry* 289 (21). American Society for Biochemistry and Molecular Biology Inc.: 14750–61. doi:10.1074/jbc.M114.551994.

[36] Ferreira, Rua Valdemar, Kaluce Gonçalves De Sousa Almondes, Greisse Viero Da Silva Leal, Silvia Maria Franciscato Cozzolino, Sonia Tucunduva Philippi, and Helen De Carvalho Rondó. 2010. "O Papel Das Selenoproteínas No Câncer." *Rev Assoc Med Bras* 56 (4): 484–88. doi:10.1590/S0104-42302010000400025.

[37] Silva Junior, E. C., L. H.O. Wadt, K. E. Silva, R. M.B. Lima, K. D. Batista, M. C. Guedes, G. S. Carvalho, et al. 2017. "Natural Variation of Selenium in Brazil Nuts and Soils from the Amazon Region." *Chemosphere* 188. Elsevier Ltd: 650–58. doi:10.1016/j.chemosphere.2017.08.158.

[38] Rayman, Margaret P. 2008. "Food-Chain Selenium and Human Health: Emphasis on Intake." *British Journal of Nutrition*. doi:10.1017/S0007114508939830.

[39] Technol, Food. 2008. "Meta-Análise Do Teor de Selênio Em Castanha-Do-Brasil." *Brazilian Journal of Food Technology* 11 (1): 54–62.

[40] Ferreira, Karla Silva, José Carlos Gomes, Carlos Roberto Bellato, and Cláudio Pereira Jordão. 2002. "Concentrações de Selênio Em Alimentos Consumidos No Brasil." *Revista Panamericana de Salud Publica/Pan American Journal of Public Health* 11 (3): 172–77. doi:10.1590/S1020-49892002000300006.

[41] Huguenin, Grazielle V B, Annie S B Moreira, Tatiana D Siant'Pierre, Rodrigo A Gonçalves, Glorimar Rosa, Glaucia M M Oliveira, Ronir R Luiz, and Eduardo Tibirica. 2015. "Effects of Dietary Supplementation with Brazil Nuts on Microvascular Endothelial Function in Hypertensive and Dyslipidemic Patients: A Randomized Crossover Placebo-Controlled Trial." *Microcirculation (New York, N.Y. : 1994)* 22 (8): 687–99. doi:10.1111/micc.12225.

[42] Huguenin, Grazielle V.B., Glaucia M.M. Oliveira, Annie S.B. Moreira, Tatiana D. Saint'Pierre, Rodrigo A. Gonçalves, Alessandra R. Pinheiro-Mulder, Anderson J. Teodoro, Ronir R. Luiz, and Glorimar Rosa. 2015. "Improvement of Antioxidant Status after Brazil Nut Intake in Hypertensive and Dyslipidemic Subjects." *Nutrition Journal* 14 (1). BioMed Central Ltd. doi:10.1186/s12937-015-0043-y.

[43] Stockler-Pinto, M. B., D. Mafra, N. E. Farage, G. T. Boaventura, and S. M F Cozzolino. 2010. "Effect of Brazil Nut Supplementation on the Blood Levels of Selenium and Glutathione Peroxidase in Hemodialysis Patients." *Nutrition* 26 (11–12): 1065–69. doi:10.1016/j.nut.2009.08.006.

[44] Arsenyan, Pavel, Edgars Paegle, Ilona Domracheva, Anita Gulbe, Iveta Kanepe-Lapsa, and Irina Shestakova. 2014. "Selenium Analogues of Raloxifene as Promising Antiproliferative Agents in Treatment of Breast Cancer." *European Journal of Medicinal Chemistry* 87 (November). Elsevier Masson SAS: 471–83. doi:10.1016/j.ejmech.2014.09.088.

[45] Wrobel, Jagoda K., Gretchen Wolff, Rijin Xiao, Ronan F. Power, and Michal Toborek. 2016. "Dietary Selenium Supplementation Modulates Growth of Brain Metastatic Tumors and Changes the Expression of Adhesion Molecules in Brain Microvessels." *Biological Trace Element Research* 172 (2). Humana Press Inc.: 395–407. doi:10.1007/s12011-015-0595-x.

[46] Chen, Yu-Chi, K Sandeep Prabhu, Arunangshu Das, and Andrea M Mastro. 2013. “Dietary Selenium Supplementation Modifies Breast Tumor Growth and Metastasis.” *International Journal of Cancer. Journal International Du Cancer* 133 (9): 2054–64. doi:10.1002/ijc.28224.

[47] Wallenberg, Marita, Sougat Misra, Agata M. Wasik, Cristina Marzano, Mikael Björnstedt, Valentina Gandin, and Aristi P. Fernandes. 2014. “Selenium Induces a Multi-Targeted Cell Death Process in Addition to ROS Formation.” *Journal of Cellular and Molecular Medicine* 18 (4). Blackwell Publishing Inc.: 671–84. doi:10.1111/jcmm.12214.

[48] Roman, Marco, Petru Jitaru, and Carlo Barbante. 2014. “Selenium Biochemistry and Its Role for Human Health.” *Metallomics*. Royal Society of Chemistry. doi:10.1039/c3mt00185g.

[49] Brigelius-Flohé, Regina, and Anna Kipp. 2009. “Glutathione Peroxidases in Different Stages of Carcinogenesis.” *Biochimica et Biophysica Acta - General Subjects*. doi:10.1016/j.bbagen.2009.03.006.

[50] Gorrini, Chiara, Isaac S. Harris, and Tak W. Mak. 2013. “Modulation of Oxidative Stress as an Anticancer Strategy.” *Nature Reviews Drug Discovery*. doi:10.1038/nrd4002.

[51] Trachootham, Dunyaporn, Jerome Alexandre, and Peng Huang. 2009. “Targeting Cancer Cells by ROS-Mediated Mechanisms: A Radical Therapeutic Approach?” *Nature Reviews Drug Discovery*. doi:10.1038/nrd2803.

[52] Misra, Sougat, Mallory Boylan, Arun Selvam, Julian E. Spallholz, and Mikael Björnstedt. 2015. “Redox-Active Selenium Compounds—from Toxicity and Cell Death to Cancer Treatment.” *Nutrients*. MDPI AG. doi:10.3390/nu7053536.

[53] Faghfuri, Elnaz, Mohamad Hossein Yazdi, Mehdi Mahdavi, Zargham Sepehrizadeh, Mohammad Ali Faramarzi, Faranak Mavandadnejad, and Ahmad Reza Shahverdi. 2015. “Dose-Response Relationship Study of Selenium Nanoparticles as an Immunostimulatory Agent in Cancer-Bearing Mice.” *Archives of Medical Research* 46 (1). Elsevier Inc.: 31–37. doi:10.1016/j.arcmed.2015.01.002.

[54] Association of Official Analytical Chemists (AOAC). 2005. "Official methods of analysis of the Association of Official Analytical Chemists”. 18th ed. Gaithersburh, 2005.

[55] Olson, O. E.; Palmer, I. S.; Cary, E. E. 1975. “Modification of official fluorimetric method for selenium in plants.” *Journal of the Association of Official Analytical Chemists*, v. 58, n. 1, p. 117-121.

[56] Bradford, Marion M. 1976. “A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding.” *Analytical Biochemistry*72 (1–2): 248–54. doi:10.1016/0003-2697(76)90527-3.

[57] Cleasby, I.R., Nakagawa, S., 2011. “Neglected biological patterns in the residuals.” *Behav. Ecol. Sociobio*l. 65, 2361-2372. doi: 10.1007/s00265-011-1254-7.

[58] Lenth, Russell V. 2016. “Least-Squares Means: The R Package Lsmeans.” *Journal of Statistical Software* 69 (1): 1–33. doi:10.18637/jss.v069.i01.

[59] R Development Core Team, 2018. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria, Version 3.4.4..

[60] Burk, Raymond F., and Kristina E. Hill. 2015. “Regulation of Selenium Metabolism and Transport.” *Annual Review of Nutrition* 35 (1): 109–34. doi:10.1146/annurev-nutr-071714-034250.

[61] Aires, Margarida de M. 2012. “Fisiologia”. *Guanabara Koogan*, 4. ed. ISBN 978-85-277-2100-4.

[62] Vendeland, Susan C., Judy A. Butler, and Philip D. Whanger. 1992. “Intestinal Absorption of Selenite, Selenate, and Selenomethionine in the Rat.” *The Journal of Nutritional Biochemistry*3 (7): 359–65. doi:10.1016/0955-2863(92)90028-H.

[63] Lee, Eun-Hyun, Seung-Kwon Myung, Young-Jee Jeon, Yeol Kim, Yoon Jung Chang, Woong Ju, Hong Gwan Seo, and Bong Yul Huh. 2011. “Effects of Selenium Supplements on Cancer Prevention: Meta-Analysis of Randomized Controlled Trials.” *Nutrition and Cancer* 63 (8): 1185–95. doi:10.1080/01635581.2011.607544.

[64] Burk, Raymond F, Kristina E Hill, and Amy K Motley. 2003. “Selenoprotein Metabolism and Function: Evidence for More than One Function for Selenoprotein P.” *The Journal of Nutrition*133 (5 Suppl 1): 1517S–20S. doi:10.1093/jn/133.5.1517S.

[65] Sies, Helmut, and Gavin E. Arteel. 2000. “Interaction of Peroxynitrite with Selenoproteins and Glutathione Peroxidase Mimics.” *Free Radical Biology and Medicine*, 28:1451–55. doi:10.1016/S0891-5849(00)00253-7.

[66] Arteel, Gavin E., Karlis Briviba, and Helmut Sies. 1999. “Protection against Peroxynitrite.” *FEBS Letters*. doi:10.1016/S0014-5793(99)00073-3.

[67] Arteel, G E, V Mostert, H Oubrahim, K Briviba, J Abel, and H Sies. 1998. “Protection by Selenoprotein P in Human Plasma against Peroxynitrite-Mediated Oxidation and Nitration.” *Biol Chem* 379 (8–9): 1201–5. http://www.ncbi.nlm.nih.gov/pubmed/9792455.

[68] Mahn, Andrea V., Héctor M. Toledo, and Manuel H. Ruz. 2009. “Organic and Inorganic Selenium Compounds Produce Different Protein Patterns in the Blood Plasma of Rats.” *Biological Research* 42 (2): 163–73. doi:10.4067/S0716-97602009000200004.

[69] Smith, Martin L., Jody K. Lancia, Timothy I. Mercer, and C. Ip. 2004. “Selenium Compounds Regulate p53 by Common and Distinctive Mechanisms.” *Anticancer Research* 24 (3 A): 1401–8.

[70] Song, Hongjiao, Xiaomin Ren, and Ping Liu. 2015. “Distribution and Inhibition Effect of Seleno-l-Methionine on 4T1 Mouse Mammary Carcinoma.” *International Journal of Physiology, Pathophysiology and Pharmacology* 7 (2). E-Century Publishing Corporation: 76–86.

[71] Huang, Cheng, Guangyu Ding, Chengyu Gu, Jian Zhou, Ming Kuang, Yuan Ji, Yifeng He, Tadashi Kondo, and Jia Fan. 2012. “Decreased Selenium-Binding Protein 1 Enhances Glutathione Peroxidase 1 Activity and Downregulates HIF-1α to Promote Hepatocellular Carcinoma Invasiveness.” *Clinical Cancer Research* 18 (11): 3042–53. doi:10.1158/1078-0432.CCR-12-0183.

[72] Xia, Yiming, Kristina E. Hill, Daniel W. Byrne, Jiayuan Xu, and Raymond F. Burk. 2005. “Effectiveness of Selenium Supplements in a Low-Selenium Area of China.” *American Journal of Clinical Nutrition* 81 (4): 829–34. doi:81/4/829 [pii].

[73] Brigelius-Flohé, Regina, and Anna Patricia Kipp. 2013. “Selenium in the Redox Regulation of the Nrf2 and the Wnt Pathway.” *Methods in Enzymology* 527: 65–86. doi:10.1016/B978-0-12-405882-8.00004-0.

[74] Sanmartín, Carmen, Daniel Plano, Arun K. Sharma, and Juan Antonio Palop. 2012. “Selenium Compounds, Apoptosis and Other Types of Cell Death: An Overview for Cancer Therapy.” *International Journal of Molecular Sciences*. MDPI AG. doi:10.3390/ijms13089649.

[75] Davis, Cindy D., and Irons, Robert. 2005. “Are Selenoproteins important for the cancer protective effects of selenium?” *Current Nutrition & Food Science* 1 (3): 201– 214. doi: https://doi.org/10.2174/157340105774574857.

[76] Davis, Cindy D., Finley, John W. 2003. “Chemical versus food forms of selenium in cancer prevention.” *Watson, Ronald R. ed, Functional foods & nutraceuticals in cancer prevention*. Ames, 55-86.

[77] Feldman, Bernard F., Zinkl, Joseph G., Jain, Nemi C. 2000. “Schalm’s veterinary hematology.” *Philadelphia*, 5.ed. 1344 p.

[78] Combs, Gerald F. 2015. “Biomarkers of Selenium Status.” *Nutrients*. MDPI AG. doi:10.3390/nu7042209.

[79] Brigelius-Flohé, Regina, and Anna Kipp. 2009. “Glutathione Peroxidases in Different Stages of Carcinogenesis.” *Biochimica et Biophysica Acta - General Subjects*. doi:10.1016/j.bbagen.2009.03.006.

[80] Thiry, Celine, Yves Jacques Schneider, Luc Pussemier, Ludwig De Temmerman, and Ann Ruttens. 2013. “Selenium Bioaccessibility and Bioavailability in Se-Enriched Food Supplements.” *Biological Trace Element Research* 152 (1): 152–60. doi:10.1007/s12011-013-9604-0.

[81] Cominetti, C., de Bortoli, M. C., Garrido, A. B., & Cozzolino, S. M. F. (2012). “Brazilian nut consumption improves selenium status and glutathione peroxidase activity and reduces atherogenic risk in obese women.” *Nutrition Research*, *32*(6), 403–407. https://doi.org/10.1016/j.nutres.2012.05.005.

[82] Dumont, Emmie, Liesbet De Pauw, Frank Vanhaecke, and Rita Cornelis. 2006. “Speciation of Se in Bertholletia Excelsa (Brazil Nut): A Hard Nut to Crack?” *Food Chemistry* 95 (4): 684–92. doi:10.1016/j.foodchem.2005.04.004.

[83] Dumont, Emmie, Frank Vanhaecke, and Rita Cornelis. 2006. “Selenium Speciation from Food Source to Metabolites: A Critical Review.” *Analytical and Bioanalytical Chemistry*. doi:10.1007/s00216-006-0529-8.

[84] Bodó, Erzsébet Tünde, Zsolt Stefánka, Ildikó Ipolyi, Csilla Sörös, Mihály Dernovics, and Péter Fodor. 2003. “Preparation, Homogeneity and Stability Studies of a Candidate LRM for Se Speciation.” In *Analytical and Bioanalytical Chemistry*, 377:32–38. doi:10.1007/s00216-003-1941-y.

[85] Wrobel, Katarzyna, Sasi S. Kannamkumarath, Kazimierz Wrobel, and Joseph A. Caruso. 2003. “Hydrolysis of Proteins with Methanesulfonic Acid for Improved HPLC-ICP-MS Determination of Seleno-Methionine in Yeast and Nuts.” *Analytical and Bioanalytical Chemistry* 375 (1): 133–38. doi:10.1007/s00216-002-1648-5.

[86] Da Silva, Elidiane Gomes, Lidiane Raquel Verola Mataveli, and Marco Aurélio Zezzi Arruda. 2013. “Speciation Analysis of Selenium in Plankton, Brazil Nut and Human Urine Samples by HPLC-ICP-MS.” *Talanta* 110 (June): 53–55. doi:10.1016/j.talanta.2013.02.014.

[87] Thomson, Christine D., Alexandra Chisholm, Sarah K. McLachlan, and Jennifer M. Campbell. 2008. “Brazil Nuts: An Effective Way to Improve Selenium Status.” *American Journal of Clinical Nutrition* 87 (2): 379–84. doi:10.1093/ajcn/87.2.379.

[88] Wang, Huali, Jinsong Zhang, and Hanqing Yu. 2007. “Elemental Selenium at Nano Size Possesses Lower Toxicity without Compromising the Fundamental Effect on Selenoenzymes: Comparison with Selenomethionine in Mice.” *Free Radical Biology and Medicine* 42 (10): 1524–33. doi:10.1016/j.freeradbiomed.2007.02.013.

[89] Suzuki, Kazuo T. 2005. “Metabolomics of Selenium: Se Metabolites Based on Speciation Studies.” *Journal of Health Science* 51 (2): 107–14. doi:10.1248/jhs.51.107.

[90] Kornsteiner, Margit, Karl Heinz Wagner, and Ibrahim Elmadfa. 2006. “Tocopherols and Total Phenolics in 10 Different Nut Types.” *Food Chemistry* 98 (2): 381–87. doi:10.1016/j.foodchem.2005.07.033.

[91] Fairweather-Tait, Susan J, Rachel Collings, and Rachel Hurst. 2010. “Selenium Bioavailability: Current Knowledge and Future Research Requirements.” *The American Journal of Clinical Nutrition* 91 (5): 1484S–1491S. doi:10.3945/ajcn.2010.28674J.

[92] Yang, Jun. 2009. “Brazil Nuts and Associated Health Benefits: A Review.” *LWT - Food Science and Technology*. doi:10.1016/j.lwt.2009.05.019.

[93] Cardoso, Bárbara R., Graziela B.Silva Duarte, Bruna Z. Reis, and Silvia M.F. Cozzolino. 2017. “Brazil Nuts: Nutritional Composition, Health Benefits and Safety Aspects.” *Food Research International*. Elsevier Ltd. doi:10.1016/j.foodres.2017.08.036.

[94] Polavarapu, Rathnagiri, Douglas R. Spitz, Julia E. Sim, Mark H. Follansbee, Larry W. Oberley, Amir Rahemtulla, and Amin A. Nanji. 1998. “Increased Lipid Peroxidation and Impaired Antioxidant Enzyme Function Is Associated with Pathological Liver Injury in Experimental Alcoholic Liver Disease in Rats Fed Diets High in Corn Oil and Fish Oil.” *Hepatology* 27 (5): 1317–23. doi:10.1002/hep.510270518.

[95] Lemaitre, D, E Véricel, a Polette, and M Lagarde. 1997. “Effects of Fatty Acids on Human Platelet Glutathione Peroxidase: Possible Role of Oxidative Stress.” *Biochemical Pharmacology*53 (4): 479–86. http://www.ncbi.nlm.nih.gov/pubmed/9105398.

[96] Cominetti, Cristiane, Maritsa Carla de Bortoli, Eduardo Purgatto, Thomas Prates Ong, Fernando Salvador Moreno, Arthur Belarmino Garrido, and Silvia Maria Franciscato Cozzolino. 2011. “Associations between Glutathione Peroxidase-1 Pro198Leu Polymorphism, Selenium Status, and DNA Damage Levels in Obese Women after Consumption of Brazil Nuts.” *Nutrition* 27 (9): 891–96. doi:10.1016/j.nut.2010.09.003.

[97] Labunskyy, V. M., D. L. Hatfield, and V. N. Gladyshev. 2014. “Selenoproteins: Molecular Pathways and Physiological Roles.” *Physiological Reviews* 94 (3): 739–77. doi:10.1152/physrev.00039.2013.

[98] Cheng, W H, Y S Ho, B a Valentine, D a Ross, G F Combs, and X G Lei. 1998. “Cellular Glutathione Peroxidase Is the Mediator of Body Selenium to Protect against Paraquat Lethality in Transgenic Mice.” *The Journal of Nutrition* 128 (7): 1070–76. doi:10.1093/jn/128.7.1070.

[99] Bermano, Giovanna, Fergus Nicol, John A Dyer, Roger A Sunde, Geoffrey J Beckett, John R Arthur, and John E Hesketh. 1995. “Tissue-Specific Regulation of Selenoenzyme Gene Expression during Selenium Deficiency in Rats.” *The Biochemical Journal* 311 ( Pt 2: 425–30.

[100] Kipp, Anna, Antje Banning, Evert M. van Schothorst, Catherine Méplan, Lutz Schomburg, Chris Evelo, Susan Coort, et al. 2009. “Four Selenoproteins, Protein Biosynthesis, and Wnt Signalling Are Particularly Sensitive to Limited Selenium Intake in Mouse Colon.” *Molecular Nutrition and Food Research* 53 (12): 1561–72. doi:10.1002/mnfr.200900105.

[101] Suzuki, Y.; Sakai, T.; Furuta, N.. 2012. “Isolation of Selenoprotein-P and Determination of Se Concentration Incorporated in Proteins in Human and Mouse Plasma by Tandem Heparin Affinity and Size-Exclusion Column HPLC-ICPMS.” *Analytical Sciences* 28 (3): 221. doi:10.2116/analsci.28.221.

[102] Gromadzinska, et al. 2003 = Gromadzinska, J.; Wasowicz, W.; Rydzynski, K.; Szeszenia-Dabrowska, N. 2003. "Oxidative-stress markers in blood of lung cancer patients occupationally exposed to carcinogens”. *Biol Trace Elem Res*. 91: 203-215.

[103] Silva, I.L.; Montero-Montero, L.; Martin-Villar, E.; Martin-Perez, J.; Sainz, B.; Renart, J.; Simões, R.T.; Veloso, E.S.; Teixeira, C.S.; Oliveira, M.C.; Ferreira, E.; Quintanilla, M. 2017. "Reduced expression of the murine HLA-G homolog Qa-2 is associated with malignancy, epithelial-mesenchymal transition and stemness in breast cancer cells". *Scientific Reports* 7, article number: 6276.

[104] Palmer, I.S.; Herr, A.; Nelson, T. 1982. "Toxicity of selenium in Brazil nuts to rats". *Journal of Food Science – Wiley Online Library*.

[105] Vega et al., 2007. “Non-optimal levels of dietary selenomethionine alter splenocyte response and modify oxidative stress markers in female mice". *Food and Chemical Toxicology* 45: 1147-1153.

[106] Rodriguez-Sosa et al., 2013 “Effect of selenomethionine supplementation in food on the excretion and toxicity of arsenic exposure in female mice”. *Biol Trace Elem Res* 156: 279-287. doi: 10.1007/s12011-013-9855-9.

[107] Ansari, A.M.; Ahmed, A.K.; Matsangos, A.E.; Lay, F.; Born, L.J.; Marti, G.; Harmon, J.W.; Sun, Z. 2016. “Cellular GFP toxicity and immunogenicity: potential confounders in in vivo cell tracking experiments”. Stem Cell Rev and Rep. 12: 553-559.

[108] Re, Francesca; Srinivasan, Ramaprasad; Igarashi, Takehito; Marincola, Franco; Childs, Richard. 2004. “Green fluorescent protein expression in dendritic cells enhances their immunogenicity and elicits specific cytotoxic T-cell responses in humans”. Experimental Hematology. 32: 210-217.

[109] Pereira, Marina Apocalypse Nogueira; Silva Júnior, Ediu Carlos; Campidelli, Marina Leopoldina Lamounier; Guilherme, Luiz Roberto Guimarães; Carneiro, João de Deus de Souza; Pereira, Luciano José. 2017. “Quantificação de alfa e gama tocoferóis por HPLC-DAD/UV-Vis em castanha do brasil (Bertholletia excelsa)”. In: Anais do XXVI Congresso da Pós-graduação, novembro de 2017. Identificador: 413-1-162.

## 9. Attachments

**UNIVERSIDADE FEDERAL DE LAVRAS**
**PRÓ-REITORIA DE PESQUISA**
COMISSÃO DE ÉTICA NO USO DE ANIMAIS
Cx.P.3037 - Lavras – MG – 37200-000 – (35) 3829-5182 cba@nintec.ufla.br

**CERTIFICADO**

Certificamos que a proposta intitulada "Efeito da suplementação de diferentes fontes dietéticas de selênio na progressão de tumor mamário induzido por inoculação de células 4T1/GFP", protocolo nº 079/16, sob a responsabilidade de Luciano José Pereira, Andressa Naira de Jesus Pereira, Marina Apocalypse Nogueira Pereira e Linaena Méricy da Silva, que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto homem), para fins de ensino e/ou pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas edificadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), do Ministério da Ciência, Tecnologia e Inovação (MCTI), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Pró-Reitoria de Pesquisa/UFLA, em reunião de 15/12/2016.

Vigência da autorização: de 01/05/2016 a 01/05/2018
Finalidade: ( ) Ensino (x ) Pesquisa Científica
Espécie/linhagem/raça: Camundongo / Balb/c
Número de animais aprovados: 40
Peso/Idade: 25g / 7-8 semanas
Sexo: fêmea
Origem dos animais (documento apresentado pelo pesquisador responsável e arquivado pela CEUA): Biotério Central da Multiusuário da Universidade Federal de Lavras - Responsável: Christiane Malfitano

Prof. Juliano Vogas Peixoto

Presidente da Comissão de Ética no Uso de Animais CEUA

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## CERTIFICATE

We certify that the proposal entitled "Effect of diets supplemented with different selenium sources in breast tumor progression induced by 4T1/GFP cells inoculation" Protocol No. 079/16, under the responsibility of Luciano José Pereira, Andressa Naira de Jesus Pereira, Marina Apocalypse Nogueira Pereira and Linaena Méricy da Silva, which involves the production, maintenance and / or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except man), for purposes of teaching and / or scientific research, is in accordance with the provisions of Law No. 11.794, of October 8, 2008, Decree No. 6899 of July 15, 2009, and with the standards required by the National Council Animal Experimentation Control (CONCEA), the Ministry of Science, Technology and Innovation (MCTI), and was approved by ETHICS COMMITTEE ON ANIMAL USE (CEUA) of the Dean of Research / UFLA in meeting 12/15/2016.

Authorization validity: 5/01/2016 to 5/01/2018
Finality: ( ) Teaching (x ) Scientific research
Species / strain / breed: Camundongo / Balb/c
Number of approved animals: 40
Weight / Age: 25g / 7-8 weeks
Sex: female
Origin of animals (document presented by the responsible researcher and filed by CEUA): Biotério Central da Universidade Federal de Lavras - Responsável: Biotério Central da Multiusuário da Universidade Federal de Lavras - Responsável: Christiane Malfitano

Prof. Juliano Vogas Peixoto

Presidente da Comissão de Ética no Uso de Animais CEUA

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