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Interaction between green tea and metformin and its effects on oxidative stress and inflammation in overweight women: a randomised clinical trial

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Abstract

This study evaluated the effect of green tea extract and metformin and its interaction on markers of oxidative stress and inflammation in overweight women with insulin resistance. After screening, 120 women were randomly allocated in 4 groups: Placebo (PC): 1g of microcrystalline cellulose/day; Green tea (GT): 1 g (558 mg polyphenols) of standardized dry extract of green tea/day and 1 g of placebo/day; Metformin (MF): 1 g of metformin/day and 1 g of placebo/day; Green Tea and Metformin (GTMF): 1 g (558 mg polyphenols) and 1 g of metformin/day. All groups were followed-up for 12 weeks with assessment of oxidative damage to lipids and proteins, specific activity of antioxidant enzymes and inflammatory cytokine serum levels. The association of green tea with metformin significantly reduced IL-6 (GTMF: −29.7((−62.6)–20.2))($p = 0.004$). Green tea and metformin isolated reduced TNF-α (GT: −12.1((−18.0)–(−3.5)); MF: −24.5((−38.60)–(−4.4)) compared to placebo (PB: 13.8 (1.2–29.2))($P < 0.001$). Also, isolated metformin reduced TGF-β (MF: −25.1((−64.4)–0.04)) in comparison to placebo (PB: 6.3((−1.0)–16.3))($p = 0.038$). However, when combined, their effects were nullified either for TNF-α (GTMF: 6.0((−5.7)–23.9) and for TGF-β (GTMF: −1.8((−32.1)–8.5). This study showed that there is a drug-nutrient interaction between green tea and metformin that is dependent on the cytokine analyzed.

Keywords: **Green tea: Metformin: Insulin resistance: Overweight: Inflammation: Oxidative stress**

In recent decades, an exponential increase in obesity has been observed[1]. Adipose tissue hypertrophy, common in obesity, promotes increased macrophage infiltration, leading to greater expression of pro-inflammatory adipokines[2,3] and increasing oxidative stress[4]. Consequently, there is also an increase in lipotoxicity and visceral fat deposition in organs such as the pancreas and liver, which can lead to the development of insulin resistance and comorbidities such as diabetes, dyslipidaemia and hypertension[3,5,6].

In addition to the already consolidated strategies for obesity treatment such as lifestyle changes associated or not with the use of medicines[7], the use of herbal medicines has also been adopted[8]. Among medicinal herbs, one of the most used is green tea (GT), a source of epigallocatechin-3-gallate.

Some studies have shown that the consumption of GT promotes the improvement of obesity and insulin resistance, in addition to the reduction of inflammatory and oxidation markers such as IL-6 and malondialdehyde, besides an increase of the antioxidant response such as the expression of superoxide dismutase[9–11]. On the other hand, a systematic review of randomised clinical trials with healthy or unhealthy adults showed that despite the well-known antioxidant properties of GT, there is no consensus on its role in oxidative stress[12].

Besides medicinal plants and drugs already recommended for the treatment of obesity, other drugs have been studied regarding their applicability for controlling obesity and insulin resistance, such as metformin (MF)[13]. MF is a drug of the biguanide class, registered for the treatment of type 2 diabetes mellitus. However, its off-label use for the treatment of obesity and insulin resistance has also become common[14,15].

MF when used in animals inhibited the expression of IL-6 and IL-1β[16]. However, its impact on serum inflammation markers in

Abbreviations: AMPK, 5'-monophosphate-activated protein kinase; GT, green tea; GTMF, green tea and metformin; MDA, malondialdehyde; MF, metformin; TGF-β, transforming growth factor beta..

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humans remains controversial[17]. In a study with overweight subjects and impaired glucose tolerance, the use of MF significantly decreased IL-6; meanwhile, a systematic review with meta-analysis evidenced no significant changes in IL-6 levels in women with polycystic ovaries[17]. Regarding side effects, MF can cause gastrointestinal disorders like diarrhoea, nausea/vomiting, abdominal pain, flatulence and other symptoms[18].

Therefore, considering that GT is one of the most consumed beverages in the world[19] due to its effects on the improvement of obesity[20] and that MF has also been recognised as a potential great therapy for that condition[21], it can be hypothesised that the combined use of these substances could be beneficial. However, in a randomised clinical trial carried out by our research group, GT presented a superior hypoglycaemic effect when compared with MF and had its effect nullified when consumed with that drug[22].

Regarding inflammation and oxidative stress control, which are factors that contribute to the development of comorbidities associated with obesity, such as diabetes *mellitus*, to the best of our knowledge, there are no studies comparing the effects of the consumption of green tea and metformin (GTMF). Thus, the aim of this study is to evaluate the effect of GT extract and MF and its interaction on markers of oxidative stress and inflammation in overweight women with insulin resistance.

Experimental methods

Material

The capsules of GT extract, MF and cellulose microcrystalline were manipulated and encapsulated in a specialised laboratory (Nathupharmus®). The analysis of the GT extract was carried out by a pharmaceutical quality control laboratory (SM Empreendimentos Farmacêuticos LTDA) and disclosed the presence of 55·75 % of total polyphenols. This data and other information obtained by the extract manufacturer (Pharmanostra®) are presented in online Supplementary Table 2.

Each GT capsule contained 500 mg of *Camellia sinensis* leaf extract (extracted with ethanol and water), with 279 mg polyphenols. MF and placebo (microcrystalline cellulose) were provided in capsules containing 500 mg of the respective substances. The amount of GT extract was chosen based on a literature review in which it was observed that the dose of catechins used in the studies varied from 208 mg to 900 mg[23] and that catechins account for about 60–80 % of the total polyphenols in tea[24,25]. Therefore, in order to obtain a reasonable amount of phenolics in a viable quantity of capsules, we chose the dose of 558 mg of total polyphenols, which were divided into two capsules.

Study design

This was a 12-week randomised, double-blind, clinical trial conducted with 120 patients from nutritional primary care facilities and the endocrinology clinic at the Clinical Hospital of the Federal University of Goias, Brazil, between June and August 2014[22].

Ethical approval

The study was registered at the Brazilian Registry of Clinical Trials (ReBEC – ensaiosclinicos.gov.br) under the number RBR-4bdwxs. The study was conducted according to the guidelines laid down in the Declaration of Helsinki and Good Clinical Practice guidelines. All procedures involving human subjects were approved by the Ethics Committee of the Federal University of Goias (reference no. 636.652). All subjects provided written informed consent to participate in this study. This study followed the Consolidated Standards of Reporting Trials for clinical studies.

Subjects

Initially, 191 participants were screened by a standardised questionnaire and laboratory tests aiming to confirm if they had insulin resistance. Methodological details were presented in the previous publication of this group[22].

The standardised questionnaire consisted of questions that addressed the eligibility criteria, namely: women aged 20–60 years with abnormal glucose concentrations evaluated by fasting blood glucose > 100 mg/dl or glycated haemoglobin ≥ 5·7 % and BMI > 28·9 kg/m^2 or BMI > 27·5 kg/m^2 and Homeostatic Model Assessment for Insulin Resistance > 3·60[26]. All biochemical parameters were assessed at the beginning of the study through blood tests. Exclusion criteria were as follows: being in the use of insulin or any other glycaemia-lowering medication, presenting previous diabetes diagnosis, kidney disease, liver disease, heart disease and hyperthyroidism; being at any weight control treatment; being pregnant, breastfeeding or at hormone therapy for menopause; and doing daily consumption of any kind of tea.

Experimental protocol

After screening, the 120 selected individuals were randomly allocated in four intervention groups using the unweighted pair group method with arithmetic mean[27]: *placebo:* four capsules containing microcrystalline cellulose/d (2 g/d); *GT:* two capsules containing standardised dry extract of GT/d (1 g/d; 558 mg polyphenols) and two capsules containing placebo (1 g/d); *MF:* two capsules containing MF (1 g/d) and two capsules containing placebo (1 g/d); and *GTMF:* two capsules containing standardised dry extract of GT/d (1 g/d; 558 mg polyphenols) and two capsules containing MF/d (1 g/d).

Unweighted pair group method with arithmetic mean is a simple agglomerative hierarchical clustering method (from bottom to top). It builds a rooted tree (dendrogram) that reflects the structure present in a pairwise similarity matrix (or dissimilarity matrix), so it was possible to randomise participants into four groups. For this purpose, R software was used on a secure computer. The groups were initially named by random letters. Only after statistical analysis, the blinding code was provided to the researchers.

An independent research group not involved in the study was responsible for administering the supplements according to the randomisation protocol. Each participant received two pots, one with red capsules and the other with blue capsules. Each colour represented a different compound. However, all participants

received the same colours, so it was not possible to identify the compound. Furthermore, all the capsules and pillboxes were the same size and were provided to participants by an independent research group according to the randomisation protocol. Participants were instructed to take one red and one blue capsule, 5 min before breakfast, and one red and one blue, 5 min before lunch. Thus, the blindness of the study occurred to participants and to researchers.

In order to assess the adherence to treatment, participants should bring the remaining capsules when returning to the laboratory, so they could be counted. Weekly short messages on cell phones and phone calls were sent to participants to increase the compliance about the consumption of the capsules.

Participants were instructed to maintain their regular lifestyle during the 12-week intervention. To ensure that these instructions were followed, a 3 d food record questionnaire based on weekdays and weekend days was applied to ensure compliance with the consumption of foods at the beginning and end of the study. Data on food records were evaluated by the research team to check if dietary information was complete and accurate, and then the records were analysed by Nutriquanti® (2011).

Biochemical analyses

Blood samples were collected from a peripheral vein after a 12 h overnight fasting at baseline and after 12 weeks of intervention. Volunteers were oriented not to drink alcohol until 72 h before blood collection, so as not to do vigorous exercises during the 24 h before the procedure. Volunteers who did not follow the guidelines had their blood collections and other tests scheduled for a later date.

Samples were centrifuged at 1560 ***g*** for 5 min at room temperature. Plasma was removed into a new tube, and cell pellets from blood samples were lysed by the addition of a buffer solution containing 0·32 M sucrose, 10 mM Tris-HCl pH 7·4, a cocktail of protease inhibitors, 5 mM $MgCl_2$ and 1 % Triton X-100. The aliquots were stored at –80°C for further determination of redox response and inflammatory markers.

Oxidative damage to lipids and proteins

Serum malondialdehyde (MDA), a product of lipid peroxidation, was measured according to the methodology described by Candan and Tuzmen[28] with adaptations. Fluorimetric analysis with excitation wavelengths at 532 nm and emission at 553 nm (SpectraMax M3 Multi-Mode Microplate Reader, Molecular Devices) was employed to determine MDA concentration by the MDA-thiobarbituric acid complex. Initially, a standard curve was obtained by hydrolysing 1,1,3,3 tetraethoxypropane (TEP, Sigma) in 1 % H_2SO_4 in the concentration range of 0·3125 to 5·05 nmol/ml ($y = 0{\cdot}0095x + 0{\cdot}0125$; $R^2 = 0{\cdot}9997$). In order to form the MDA–thiobarbituric complex, 150 µl of serum was diluted on 450 µl of Milli-Q water. The homogenate was centrifuged at 12 000 ***g*** at 4°C for 5 min.

Subsequently in an Eppendorf tube, 375 µl of 440 mmol/l phosphoric acid (H_3PO_4) and 125 µl of 42 mmol/l thiobarbituric acid were mixed with 250 µl of the supernatant of the serum diluted sample. Then, the sample was incubated at 99°C for 1 h in a thermomixer device (Thermomix Comfort, Eppendorf). After the complex was formed, tubes were cooled, and 600 µl of that sample was added to 600 µl of a precipitation solution (methanol: 1 M NaOH at a proportion of 91:9 v/v). Samples were centrifuged at 12 000 ***g*** at 4°C along 5 min, the supernatant was removed and the readings were performed in a spectrofluorometer (Spectramax M2) with emission at 553 nm and excitation monitored at 532 nm. The results were expressed as nmol MDA/ml serum.

Carbonylated protein concentration in the serum was assessed as a method described by Richert *et al.*[29], which consists in monitoring the absorbance of the complex dinitrophenylhydrazine-carbonyl groups at 376 nm (Spectrophotometer, Shimadzu – TCC 240A). The measured concentration of protein carbonyl was expressed as µmol protein carbonyl groups/mg total protein using an extinction coefficient of 22·000 mM^{-1}/cm. The method previously described by Hartree[30] was employed to determine total serum protein.

Reactive oxygen metabolites measured by the Diacron-reactive oxygen metabolites

The *Diacron-reactive oxygen metabolites* test was carried out according to the methodology proposed by Ito *et al.*[31]. In order to analyse the total amount of hydroperoxides in serum, samples were placed in microplates filled with a buffer and N,N-diethyl-p-phenylenediamine, a colour-developing chromogen, was added and mixed in, resulting in the chromogen substrate being oxidised by the free radicals. The colour change in the plate was measured in a spectrophotometer at 550 nm and 37°C and recorded as Carratelli units, named as its inventor, which is equivalent to 0·08 mg/Dl of H_2O_2[32,33].

Specific activity of antioxidant enzymes

A suspension of 0·05 µg protein/µl (20 µl) was added to a microplate with 140 µl of phosphate buffer (50 mM with 0·1 mM EDTA, potential hydrogen (pH) 7·4) and 40 µl of 30 mM of freshly prepared hydrogen peroxide. The 240 nm absorbance was monitored continuously for 8 min at 30°C. A standard curve was prepared using the catalase enzyme (0·44–14·17 U/mg protein)[34].

Regarding superoxide dismutase, analysis was performed as described by Ewing and Janero[35] with modifications. An erythrocyte suspension containing 0·024 µg protein /µl (25 µl) has been added to a plate containing 200 µl of a freshly prepared solution of 0·1 mM EDTA, 62 µM nitrotetrazolium blue chloride and 98 µM of NADH to 50 mM phosphate buffer (pH 7·4). The reaction was initiated with the addition of 25 µl of freshly prepared 33 µM phenazine methosulfate in a 50 mM phosphate buffer (pH 7·4) containing 0·1 mM EDTA. Absorbance at 560 nm was continuously monitored from 5 min until the nitrotetrazolium blue chloride reduction index. A standard curve (0·17–2·77 U/mg protein) was prepared using the SOD kit (Millipore Sigma, Sigma Aldrich).

Inflammatory cytokine serum levels

Serum levels of IL-1β (no.: RAB0273), IL-6 (no.: EZIL6), IL-10 (no.: RAB0244), IL-12 (no.: RAB0254), monocyte chemoattractant protein-1 (no.: EZMCP1), transforming growth factor beta (TGF-β) (no.: RAB0461) and TNF-α (no.: EZHTNFA-150K) cytokines were quantified in ng/ml by enzyme-linked immunosorbent MCP assay using commercial kits (Millipore Sigma, Sigma Aldrich). Briefly, samples were placed in microplates previously sensitised with antibodies specifically for each of the cytokines and then incubated.

After initial washing detection antibodies were added, and the samples were incubated. Subsequently, they were incubated for 15 min at darkness with 12 µl of antibody detection, 48 µl of the correspondent enzyme and 1200 µl of fetal bovine serum and completed with phosphate-buffered saline. That solution was discarded, and 50 µl of the detection solution with an enzyme was added and maintained for 60 min, followed by further washing and incubation with the substrate.

After a period of 30 min, a stop solution was added to the wells to stop the reaction. The absorbance values for each sample were obtained by reading out in a spectrophotometer at 450 nm and 670 nm. A standard curve was obtained for each cytokine provided by the manufacturer in the following concentration ranges: IL-1β (3·9–250 pg/ml), IL-6 (3·69–300 pg/ml), monocyte chemoattractant protein-1 (31·25–2000 pg/ml), TGF-β (125–8000 pg/ml), IL-10, IL-12 and TNF-α (7·8–500 pg/ml).

Statistical analyses

This study was a continuation of a previously reported randomised, double-blind clinical trial[22], whose primary outcome was glycated haemoglobin and secondary outcomes were lipid profile, body composition, fasting blood glucose, inflammatory cytokines and markers of oxidative stress. Therefore, the sample size calculation was carried out based on the primary outcome. The estimated effect size was set to a 1 % decrease from the values in the control group. By considering a significance level of 0·05, a variance of 1·5 (%) and a power of 0·8, the sample size was estimated to be twenty-four subjects in each group. A probability of a 25 % dropout rate was considered; thus, the study was initiated with 120 subjects. Data regarding lipid and glycaemic profile and body composition were previously published in Ferreira *et al.*[22]. Therefore, in this current manuscript, only markers of inflammation and oxidative stress will be presented.

Descriptive statistics (median and interquartile range (IQR)) were calculated for each variable in each group for baseline (T0), after treatment (T90) and in the percentage of intra-group variation. The Shapiro–Wilk normality test was used to test the distribution of variables. Kruskal–Wallis followed by Dunn's *post hoc* test was carried out to compare the percentage of intra-group variation between groups. All tests considered bilateral hypotheses and a level of significance of 5 % ($P < 0·05$). Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 22·0 programme.

Results

Participant characteristics

Initially, 191 women were enrolled: 153 subjects met the study inclusion criteria, and 120 agreed to participate in the study. The subjects were randomly assigned to one of four intervention groups (Fig. 1). Thirty-two women withdrew during the intervention: fourteen for personal reasons, nine due to lack of contact and nine because of inadequate capsule intake. As a result, eighty-eight participants completed the study (Fig. 1). At baseline, all groups had homogeneous characteristics, except for age in the GT group ($P = 0·049$) (online Supplementary Table 1).

Oxidation markers and antioxidant enzymes

No significant difference was observed between groups for oxidation markers and antioxidant enzymes after 12 weeks of treatment (Table 1).

Inflammatory cytokine serum levels

A significant difference between groups was observed for IL-1β ($P = 0·002$) and IL-10 ($P = 0·006$). However, this difference was not detected after the *post hoc* test, and all groups were similar to placebo. Green tea, only when associated with MF, significantly reduced IL-6 (GTMF (ng/ml): –29·7 ((–62·6)–20·2)) throughout the 12 weeks of treatment compared with placebo ($P = 0·004$). GTMF alone reduced TNF-α (GT (ng/ml): –12·1 ((–18·0)–(–3·5)); MF (ng/ml): –24·5 ((–38·60)–(–4·4)) compared with placebo (PB (ng/ml): 13·8 (1·2–29·2)) ($P < 0·001$). However, their effects were nullified when administered together (GTMF (ng/ml): 6·0 ((–5·7)–23·9). MF treatment promoted a reduction in TGF-β (MF (ng/ml): –25·1 ((–64·4)–0·04)) in comparison with placebo (PB (ng/ml): 6·3 ((–1·0)–16·3)) ($P = 0·038$). But, when combined with GT, that effect was also not observed (GTMF (ng/ml): –1·8 ((–32·1)–8·5) (Table 2).

Discussion

To our knowledge, this was the first study to compare the effects of the consumption of GTMF on inflammation and oxidative stress in humans. This study showed that GT alone reduced TNF-α, while MF not only reduced TNF-α but also TGF-β. However, these effects were nullified when they were administered together. Moreover, the combination of GTMF reduced IL-6.

It is well-known that obesity is associated with a chronic state of low-grade inflammation, characterised by progressive infiltration by cells of the immune system and an increased expression of inflammatory cytokines[36], as observed in the placebo group. Moreover, adipose tissue inflammation is a predictive factor for the development of insulin resistance in people with obesity[37], once TNF-α impaired insulin signalling in adipose tissue by suppression of insulin receptor substrate 1[38].

In our study, the consumption of GT and MF, both isolated, decreased TNF-α concentration. Both MF[39] and epigallocatechin-3-gallate from GT[40] attenuate inflammation through the

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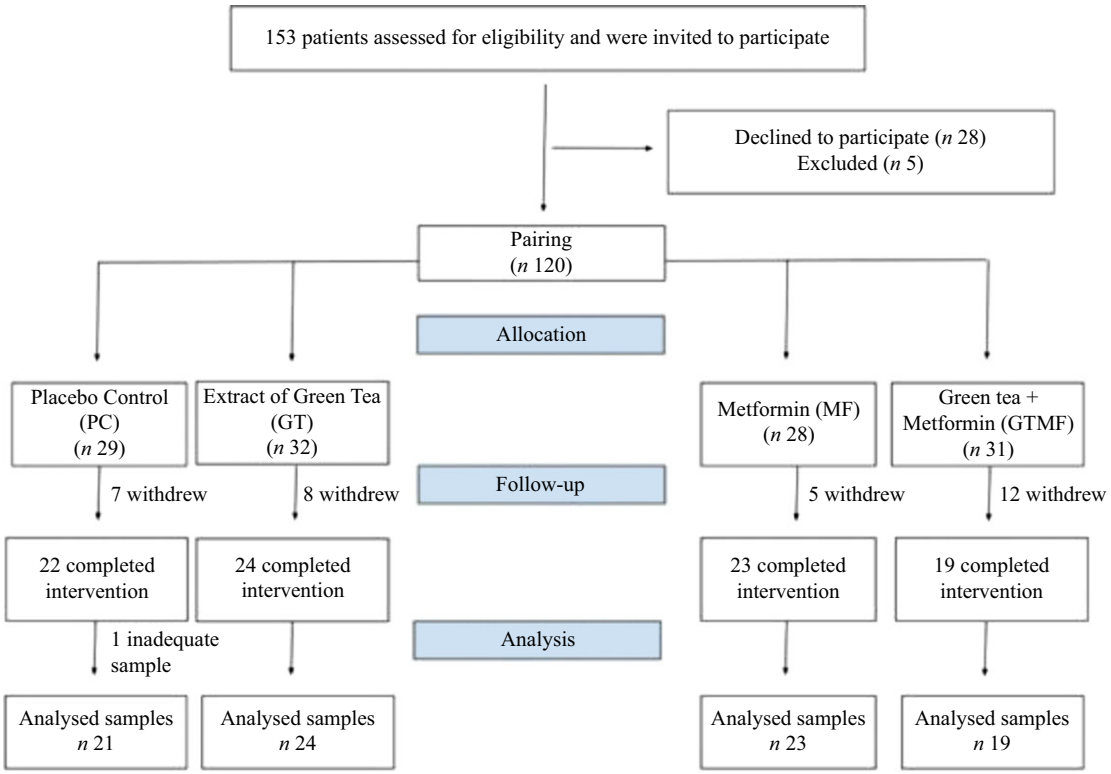

Fig. 1. Flow chart of the allocation of participants along the study.

activation of the adenosine 5'-monophosphate-activated protein kinase (AMPK). Zhao *et al.*[41] demonstrated that exposure of lipopolysaccharide-stimulated neutrophils to AMPK agonist inhibited the release of TNF-α and IL-6, which justifies the reduction of these cytokines in the groups that received either compound alone. In addition, AMPK activation, by reducing TNF-α, enhanced the insulin pathway in adipocytes and, consequently, the glycaemic control in the GT group, as evidenced in our previously published study[22].

This reduction in TNF-α levels observed in the groups treated with either GT or MF alone was nullified when both substances were administered together, demonstrating the presence of a drug–bioactive compound interaction. Considering that both compounds can interact with AMPK, the antagonistic effect was probably due to competition for the same target of action.

As a result, TNF-α impaired insulin signalling in adipose tissue by suppression of insulin receptor substrate 1[38], nullifying the hypoglycaemic effect observed in the GT group as described by Ferreira *et al.*[22]. Moreover, considering that GT extract is a mixture of several compounds and that both GT extract and MF share the same mechanism of action, it can be hypothesised that administering GT with MF alters their bioavailability. This alteration may occur through complexation via covalent bonding or other types of interactions between them[42], leading to an annulment of the reduction in TNF-α concentration observed when the products are used separately.

Another interaction occurred in the IL-6 signalling pathway, where a reduction was observed in the group that received both compounds. In our study, the action of GT on AMPK seems to prevail over MF. In addition, Krausova *et al.*[43] demonstrated a suppressive effect of MF on pregnane X receptor and other ligand-activated nuclear receptors in the transactivation of the main detoxification enzyme cytochrome P450 family 3 subfamily A member 4 in human hepatocytes. The enzymatic inhibition of systems such as cytochrome P450 family 3 subfamily A member 4 can slow down the biotransformation of the medicine itself and others that have been administered simultaneously[44,45]. As GT had a greater effect on AMPK and the MF can inhibit cytochrome P450 family 3 subfamily A member 4, the combined administration of both compounds may have led to the slowing of GT's biotransformation contributing to enhancing the reduction in IL-6 observed with isolated intake.

MF also reduced TGF-β. The TGF-β family has more than thirty-three components, including TGF-β1, TGF-β2 and TGF-β3[46]. The reduction found for TGF-β in the group treated with MF in our study endorses literature[47]. MF demonstrates antifibrotic capacity, important in visceral adipose tissue, through a mechanism that promotes the activation of AMPK and the suppression of TGF-β/SMAD family member 3[48]. Another mechanism related to the activation of AMPK by MF is the activation of the reducing receptor for advanced glycation end products and the suppression of NF-kB. This leads to a decrease in the expression of inflammatory cytokines such as TGF-β[48,49].

As strengths of this study, we can mention the robust design and innovative theme with contributions to clinical practice and as for limitations, the lack of evaluation of the catechin content present in GT extract. Moreover, the high dropout rate may have

Table 1. Effect of isolated green tea, isolated metformin and green tea + metformin consumption on the oxidative damage, reactive oxygen metabolites, specific activity of antioxidant enzymes profile of women with overweight and insulin resistance (Median values and interquartile ranges)

	Placebo						Green tea						Metformin						Green tea + metformin						
	T0		T90		%		T0		T90		%		T0		T90		%		T0		T90		%		
	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	*P*
MDA (nmol/ml plasma)	11·5	10·8–14·0	11·7	10·0–14·3	–3·1	–16·0–32·5	12·5	10·5–13·7	12·6	10·6–14·7	–1·2	–20·0–27·4	11·8	10·3–12·5	12·0	10·5–13·2	0·9	–12·9–7·6	11·5	9·7–13·8	11·5	10·4–12·9	–0·4	–15·0–21·2	0·841
Protein carbonyl (μmol/mg protein)	119·0	98·5–147·0	119·5	95·8–146·0	3·4	–24·9–33·9	107·0	85·5–144·0	121·5	106·3–149·5	12·9	–14·5–55·6	110·0	84·0–117·0	116·0	100·5–131·0	12·5	–15·3–35·6	114·0	97·0–152·5	122·5	97·0–137·5	5·5	–10·5–17·5	0·61
CAT (U/mg protein)	3687·2	3522·7–3997·8	4800·2	4308·3–5039·7	21·9	11·9–41·8	3778·9	3416·3–4150·1	4816·3	4636·9–5169·4	29·1	19·8–44·4	4038·9	3607·3–4465·0	4903·5	4197·5–5019·2	16·9	–0·7–38·2	4021·8	3878·5–4422·0	5110·1	4556·9–5324·1	28·0	1·1–33·1	0·235
SOD (U/mg protein)	7·3	5·7–8·9	6·7	5·8–7·6	–15·6	–28·9–24·5	5·8	5·1–7·0	6·7	6·0–7·8	23·1	–16·9–51·3	5·9	4·8–7·8	6·2	5·3–7·0	–6·3	–29·3–29·3	6·3	5·2–7·8	6·6	6·0–7·6	11·7	–19·2–34·2	0·233
d-ROMs (U. CARR)	86·9	79·3–116·5	85·8	75·2–107·1	–3·0	–18·7–11·0	89·5	81·2–108·3	84·7	73·2–108·1	–5·0	–15·2–7·7	94·0	66·7–111·5	73·1	59·7–84·4	–12·5	–29·5–6·5	91·2	79·9–104·2	85·9	60·4–94·7	–18·1	–27·1–1·9	0·169

MDA, malondialdehyde; CAT, catalase; SOD, superoxide dismutase; d-ROMs, *Diacron-reactive oxygen metabolites*; U. CARR, Carratelli units; T0: values at baseline. T90: values after 90 d of treatment. Data correspond to median and interquartile ranges (percentile 25 % – Q1 and percentile 75 % – Q3). *P*: statistical differences between the percentage of difference in the T0–T90 values of the control and treatment groups.

Table 2. Effect of isolated green tea, isolated metformin and green tea + metformin consumption on the serum cytokines profile of women with overweight and insulin resistance (Median values and interquartile ranges)

	Placebo						Green tea						Metformin						Green tea + metformin						
	T0		T90		%		T0		T90		%		T0		T90		%		T0		T90		%		*P*
Cytokine (ng/ml)	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median	IQR	Median
Il-1β	11·46	85·15–19·38	14·63	11·26–17·24	–0·6	–24·1–15·2^{AB}	11·28	5·40–18·65	6·57	4·18–17·09	–6·1	–21·8–6·1^{A}	5·42	3·32–10·72	9·98	5·20–14·71	46·3	2·3–102·5^{B}	15·35	11·93–27·82	11·99	8·73–19·14	–4·9	–21·1–26^{AB}	0·002
IL-6	12·63	8·26–17·41	16·99	12·78–20·92	14·2	6·6–42·1^{B}	13·03	6·08–22·82	7·50	3·79–22·84	–6·1	–39·2–9·8^{AB}	11·52	7·41–15·66	14·53	9·26–22·88	15·1	–21·1–89·2^{B}	14·27	8·14–21·21	10·05	6·71–19·29	–29·7	–62·6–20·2^{A}	0·004
IL-10	14·61	10·78–16·83	15·29	14·48–18·13	10·8	–2·7–25·1^{AB}	9·66	5·23–18·80	9·09	5·87–15·66	8·1	–21·0–46·1^{AB}	13·02	9·90–14·74	10·60	7·82–12·47	–21·6	–34·3–7·5^{A}	9·70	4·34–13·86	12·31	7·16–17·09	26·1	8·2–103·9^{B}	0·006
IL-12	98·98	60·44–156·80	43·64	24·60–61·85	–50·5	–70·1–(–13·2)	97·61	66·87–154·98	66·62	44·38–90·24	–57·0	–68·0–(–6·3)	110·67	58·76–233·23	83·95	60·06–150·24	–42·3	–56·1–(–4·4)	32·88	20·52–55·07	33·55	24·24 –45·51	–0·4	45·2–28·4	0·127
MCP-1	58·44	43·12–67·35	61·12	57·50–72·53	10·8	–2·7–25·1	38·64	20·92–75·20	36·39	23·50–62·65	8·1	–21·0–46·1	52·11	39·99–59·27	42·76	32·15–50·99	–19·1	–31·7–7·7	43·12	19·77–70·53	60·36	28·91–69·54	10	–11·8–36·7	0·059
TNF-α	31·73	25·06–38·20	38·30	30·65–44·35	13·8	1·2–29·2^{B}	20·93	16·83–24·02	18·41	17·06–21·53	–12·1	–18·0–(–3·5)^{A}	16·45	13·88–18·44	11·94	9924·2–16 269·2	–24·5	–38·6–(–4·4)^{A}	15·94	9·22–23·58	12·35	9·34–20·45	6·0	–5·7–23·9^{AB}	<0·001
TGF-β	952·28	735·26–1305·10	1156·05	922·05–1357·64	6·3	–1·0–16·3^{B}	957·15	568·51–1200·19	879·82	602·88–1059·00	–4·2	–25·3–26·3^{AB}	689·59	437·98–1315·85	665·21	144·28–1136·20	–25·1	–64·4–0·4^{A}	61·385	372·43–1169·37	653·85	498·19–846·34	–1·8	–32·1–8·5^{AB}	0·038

MCP-1, monocyte chemoattractant protein-1; TGF-β, transforming growth factor beta. T0: values at baseline. T90: values after 90 d of treatment. Means with the same letter are not significantly different from each other. Data correspond to median and interquartile ranges (percentile 25 % – Q1 and percentile 75 % – Q3). *P*: statistical differences between the percentage of difference in the T0–T90 values of the control and treatment groups. Different superscript letters on the same line show a significant difference among groups.

impacted the results related to IL-1β and IL-10 and oxidative stress markers.

Conclusion

In conclusion, there was a drug–nutrient interaction between GT extract and MF, and the type of interaction varies depending on the cytokine analysed. Therefore, the joint use of MF and GT seems not to be recommended for their therapeutic effects on insulin resistance.

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C. O. V., M. A. F., S. A., K. G. M. and S. F. A. contributed to the investigation. E. Y. N. conducted the statistical analysis. C. O. V. conducted the data curation and drafted the manuscript. S. F. A. and P. B. B. were responsible for supervision and for the review of the manuscript. M. A. F. and P. B. B. were responsible for methodology, and P. B. B. conducted conceptualisation, resources, funding acquisition and project administration.

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Supplementary material

For supplementary material/s referred to in this article, please visit https://doi.org/10.1017/S0007114524002356.

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