Anthropometric, metabolic and immunological factors in overweight/obesity and their influence on the responsiveness to a nutritional intervention for body weight management.

Jack Warburton

This research programme was carried out in collaboration with the Institute of Food Science, Technology and Nutrition (ICTAN) of the Spanish National Research Council (CSIC), Madrid

A thesis submitted in partial fulfilment of the requirements of Liverpool John Moores University for the degree of Master of Philosophy

October 2017

Contents

Abstract

Obesity is a public health concern that presents considerable difficulty in its management. Efforts are extensive to discover and develop new strategies to reduce body weight and fat accumulation. A group of plant-derived chemicals, known as polyphenols and with recognised anti-oxidant and anti-inflammatory properties, have been suggested as potential candidates to aid weight loss and management. On the other hand, great variability is observed in the individual responsiveness to weight-loss treatments; a multitude of factors, both physiological and environmental, may contribute to this variability, but their specific contributions are not properly understood. This project aimed to explore the relationships between obesity and selected lifestyle factors, metabolic and immunological markers, and to analyse the effect of a polyphenol-based weight-loss intervention, assessing individual responsiveness and determining potential predictors of weight loss.

This project studied 79 men and women (≥18 years; 39.8% women) classified as overweight or obese, who received drinks containing polyphenol-rich extracts, in a placebo-controlled, double-blind, crossover intervention. In a first stage, the associations between anthropometric indices and the above mentioned blood factors were analysed. Baseline and post-treatment values for all variables were then compared. Finally, participants were classified into tertiles according to their relative post-treatment body weight change into high (≥1.5% initial weight loss) or low (any weight gain) respondents; those who neither lost nor gained weight were excluded from the analysis. The participants' baseline characteristics were compared, and the correlations between body weight change and the different markers studied were analysed, in order to identify potential predictors for subsequent regression analysis.

Obesity markers in our sample were linked to higher levels of metabolic syndrome and inflammation markers. The polyphenol-based treatment (B2) were associated with small but significant reductions in body mass index (BMI) in women, reductions in specific white blood cell subsets counts (white blood cell counts and neutrophil, total T helper cells ($CD4^+$), naive T helper cells ($CD4^+RA^+$) counts), increased red blood cell haemoglobin concentration and levels of circulating non-esterified fatty acids. Body fat %, anthropometric indices, and all other blood variables analysed did not change significantly in response to the treatments. There was large variability in relative body weight change post-treatment (-4.63% to + 3.93%). High respondents presented lower baseline values for the obesity indices (BMI and WHeR), T naïve ($CD8^+RA^+$) counts and interleukin-6 (IL-6). Relative body weight change was negatively correlated with blood levels of triglycerides, very low-density lipoprotein-

cholesterol (VLDL-cholesterol) and granulocyte-macrophage colony-stimulating factor (GM-CSF). A regression model including compliance with the treatment, triglycerides and BMI explained 18.1% of the variability in weight change (P=0.003). In women only, compliance and GM-CSF explained 30% of the variability (P=0.040), and in men, B cell ($CD19^+$) count and BMI explained 13.6% of the variability (P=0.047).

In conclusion, Future work is needed to study the contribution of lifestyle factors, in particular physical activity and dietary composition, to the responsiveness to the present treatment. Research in the field of personalised nutrition will allow a more streamlined approach to weight loss and contribute to manage the current obesity epidemic.

1. Introduction

1.1. Definition of obesity, diagnosis, classification and epidemiology

Obesity is the accumulation of excessive body fat to the extent that it has a negative influence on overall health (James *et al*., 2000; Ogden *et al*., 2007).

The World Health Organisation (WHO) has stated that in 2008 half a billion adults worldwide were obese (WHO, 2013). Since 1980, the prevalence of obesity throughout the world has doubled (as reviewed by Bhurosy & Jeewon, 2014). Obesity is a growing issue, not only in the developed world but also in developing countries (Bhurosy & Jeewon, 2014). The large increase in obesity prevalence is a major problem for healthcare systems throughout the world. The United Kingdom Government reported the cost accrued by the National Health Service (NHS) in England because of obesity was above £5 billion (UK Department of Health, 2015). This equates to 5% of the total NHS budget. It has been estimated that in other European countries the cost attributed to obesity and obesity-related illnesses is around 6% to 10% of total health department budgets (Finkelstein *et al*., 2009). Obesity-related spending within the United States in 2010 was estimated at $315.8 billion, accounting for 27.5% of health care expenditure (Cawley *et al*., 2014). This is a dramatic increase since 2008, where the United States health care cost for obesity-related health issues was $147 billion (Finkelstein *et al*., 2009). A study conducted by Finkelstein and co-workers (2012) predicted that in the next two decades there will be a 33% increase in the prevalence of obesity, assuming the current trends continue. In light of this increase, the strain on the global health care systems will increase. This perspective demonstrates the importance of research within this field.

There are several methods used to diagnose obesity. The most common and widely used is body mass index (BMI), which is calculated as:

$$BMI = \frac{body\ weight\ (kg)}{height\ (m)^2}$$

Adolphe Quetelet (1796–1874) first proposed this method for calculating BMI in 1832 (Eknoyan, 2008). BMI classifies individuals into four main groups according to their score (Table 1.1); these cut offs apply to populations of non-Asian origin. In research and clinical fields, there has been an increase in the use of waist circumference (WC) as a method of identifying obese and overweight individuals, particularly those with abdominal obesity (Hu, 2007) (Table 1.1). Waist circumference (Wei *et al*., 1997; Welborn & Dhaliwal, 2007) along with waist-to-hip ratio (WHR) (Jansses *et al*., 2005; Bigaard *et al*., 2005) and waist-to-height ratio (WHeR) (Ho *et al*., 2003; Ashwell & Hsieh, 2005)

provide greater insight than BMI when assessing central adiposity. Alberti and colleagues (2009) discussed that abdominal adiposity consists of visceral fat which is metabolically active with strong associations with metabolic deregulation. Indeed, abdominal obesity has presented stronger associations than BMI with markers of disease such as serum levels of inflammatory proteins (Bastard *et al.*, 2006; Hsiech *et al.*, 2007).

Table 1.1. Classification of obesity according to BMI, waist circumference, WHR and WHeR

Classification	BMI (kg/m^2)	WC (cm)		WHR		WHeR
		F	M	F	M	
Under weight	≤ 18.4	-	-	-	-	-
Healthy weight	18.5 to 24.9	<80	< 94	<0.85	<0.90	<0.5
Overweight	25 to 29.9	80 – 88	94 - 102	-	-	-
Obese	30 to 39.9	>88	>102	≥0.85	≥0.90	<0.5
Morbidly obese	≥ 40	-	-	-	-	-

Information sourced or BMI (National Health Service Choices, 2016); WC (National Insitute for Health Care Excellence, 2014); WHR (WHO, 1999); WHeR (Hsieh *et al*., 1995). BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; F: female; M: male.

1.2. Causes of obesity

It has been shown that the regulation of body weight has a significant genetic influence (Hjelmborg *et al*., 2008); however, genetics alone cannot account for the dramatic weight gain that has occurred over the past three decades which therefore, suggests a large environmental component. The culture and lifestyle of the western world promotes high consumption of energy rich foods and sedentary lifestyles (Hill & Commerford, 1996; Halton *et al*., 2006; Carrera-Bastos *et al.*, 2011). French and co-workers (2001) stated that this dietary and lifestyle pattern is also often related to increased prevalence of metabolic diseases (reviewed by Han & Lean, 2016). Over the past few decades it has been shown that a variety of internal regulators mediate the crosstalk between adipose tissue and metabolic organs, for example the liver, muscle, and the pancreas (Rosen & Spiegelman, 2006). With excess adiposity, dysfunctions in adipokine signalling pathways often result in impaired organ communications and metabolic abnormalities (Trujillo & Scherer 2006)(Table 1.2).

Table 1.2. Obesity related metabolic abnormalities

Metabolic abnormalities	Reference
Type 2 diabetes mellitus	Donini *et al.*, 2010; Kassi *et al*., 2011; Cawley and Meyerhoefer, 2012
Cardiovascular disease	Donini *et al.*, 2010; Lubrano *et al*., 2012;

The increase in the prevalence of obesity could be attributed to the spread of the western diet. Westernised diet is defined as a dietary pattern that is high in red and processed meat, refined grains, sugar and fat, and low in polyunsaturated fatty acids and fibre (Halton *et al*., 2006). This high–energy, low-fibre diet was originally observed in high-income, developed countries; however, its emergence in the developing world has greatly increased (Popkin, 2006). Alongside the spread of this westernised diet, an increase in degenerative diseases has been witnessed (reviewed by Carrera-Bastos *et al*., 2011). However, whether it is strictly the caloric content of the food consumed or the quality of the diet as well that plays a role in inducing body adiposity and metabolic abnormalities is under debate (Heinonen *et al*., 2014). Furthermore, is it the obesity that promotes the metabolic abnormalities, or the diet? Diets that have high fat contents has been shown to promote obesity (Mozaffarian *et al*., 2011), however, it is generally unknown as to whether it is the high fat content or the western diet, that is rich is fat and sugar that exacerbates the promotion of obesity and other metabolic abnormalities (Heinonen *et al*., 2014).

In addition to the spread of energy-dense food with a low fibre content, the reduction of energy expenditure due to low levels of physical activity and the promotion of a sedentary lifestyle are significant contributors to the prevalence of obesity (Bann *et al*., 2015) as a result of a positive energy balance. Energy intake exceeding energy expenditure results in an increase of body mass, of which 60 to 80% is generally body fat (Hill & Commerford, 1996). The whole world has observed a dramatic increase in the availability of energy dense foods and sedentary lifestyles. This has led to the explosion in the prevalence of obesity and other related metabolic disorders (Chaput *et al*., 2011).

Early developmental factors have also been shown to influence obesity in adulthood. A plethora of articles have provided evidence to support that early developmental factors, such as birth weight (Parlee & MacDougald, 2014), or the effect of breastfeeding (Dietz, 2001) can have a significant effect on adult susceptibility to excessive weight gain. Parlee and MacDougald (2014) discussed in detail the relationship between birth weight and adult obesity. When using birth weight

relative to parental size, a linear relationship was presented with BMI at ages seven, eleven, sixteen and twenty-three (Parsons *et al*., 2001). The same study also found that birth weight started to present a J shaped relationship with BMI from the age of thirty-three, showing both low and high birth weight were weakly correlated with subsequent obesity (Parsons *et al*., 2001). A relationship between birth weight and adult BMI, waist-to-hip ratio and body fat percentage has also been demonstrated in various other studies (Law *et al*., 1992; Dolan *et al*., 2007; Chen *et al*., 2012). The idea that both high and low birth weight can promote obesity in adult populations has created controversy. A meta-analysis of fifteen studies only found a relationship between higher birth weight and the risk of being overweight and obese (Zhao *et al*., 2012; as reviewed by Parlee & MacDougald, 2014). On the other hand, in-utero nutritional deficiency has been shown to have both a promoting and preventative effect on adult obesity, depending on which gestational period it occurs (Ravelli *et al*., 1976). Ravelli and colleagues found that individuals who were exposed to famine in the last trimester of pregnancy and the first months of life presented significantly lower BMI values, while those exposed during the first half of the pregnancy reached higher BMI when adults (Ravelli *et al*., 1976). These differential development may explain the discrepancies in the findings on the relationship between birth weight and obesity prevalence that is observed within the literature.

Breastfeeding is another early developmental factor that has been shown to have an impact on adult obesity prevalence. Breastfeeding has a vast array of beneficial effects, such as reducing the prevalence of asthma and respiratory infections (Del Giudice *et al*., 2012), atopic eczema (Salpietro *et al*., 2011), or celiac disease (Akobeng *et al*, 2006). Dietz's (2001) was one of the original studies to report a protective role of breastfeeding upon obesity prevalence in later life. A meta-analysis of 28 studies conducted in 2005 suported that breastfeeding may act as a protective agent against the development of obesity(Owen *et al*., 2005), and a study commissioned by the WHO in 2007reported that breastfeeding reduced obesity prevalence in adults by as much as 22% (Horta *et al*., 2007). Another study accounting for various maternal factors concluded that exclusive breastfeeding for 6 to 7 months of age was strongly associated with an overall reduction in prevalence of overweight and obesity in comparison to infants fed by formula (Yamakawa *et al*., 2013; as reviewed by Marseglia *et al*., 2015). The association between breastfeeding and obesity could be due to human milk's involvement in the programming of energy balance and regulation; from day to day the milk changes and influences the metabolic state of the infant, which in turn affects the BMI later in life (Dewey, 1998; Rodriguez-Palmero *et al*., 1999; as reviewed by Marseglia *et al*., 2015). In contrast to the above

studies, others did not find a protective effect of breastfeeding upon obesity in later life (Kwok *et al*., 2010; Mohammadreza *et al*., 2012). Kwok and co-workers (2010) found that in a non-European setting breastfeeding did not reduce the risk of excess adiposity in children. Cofounding with environmental factors in the non-European settings were proposed as the reason (Kwok *et al*., 2010).

1.3. Obesity related disorders: role of inflammation

Obesity is connected to various medical conditions, such as metabolic syndrome (Table 1.2). An underlying inflammatory cause has been identified as the origin of metabolic disorders (Hotamisligil, 2006). Obese individuals present higher circulating levels of inflammatory proteins, such as tumour necrosis factor alpha (TNFα), interleukin (IL) 6, or C-reactive protein; this has led to the suggestion of a relationship between obesity and inflammation (Festa *et al*., 2001; Bullo *et al*., 2003; Park *et al*., 2005; Bastard *et al*., 2006; Perez de Heredia *et al*., 2012; Schmidt *et al*., 2015). Inflammatory cytokines (Furthermore, those with abdominal obesity present significantly higher values again in comparison to those without (except TNF-α). The accumulation of excessive adiposity is linked to activation of immune cells and the establishment of an inflammatory response (Van Gaal *et al*., 2006; Esser *et al*., 2014). Elevated levels of inflammatory cytokines are the most probable link between obesity and metabolic disorders, in particular insulin resistance (Schmidt *et al.*, 2015).Cytokines such as IL-1β hold an important role in the development of insulin resistance and type-2 diabetes (Larsen *et al*., 2007). IL-1 blockade using the IL-1-receptor antagonist anakinra can be sufficient to improve glycemia and insulin secretion (Larsen *et al*., 2007). In addition to this, Winer and colleagues (2011), showed that B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. This therefore suggests a critical role of obesity related inflammation in the development of metabolic syndrome. However, it is difficult to truly confirm which proceeds the other. It is a two way road, fatty acids can trigger inflammatory actions from macrophages through binding to toll-like receptors (Pal *et al*., 2012; Huang *et al*., 2012).

The cells responsible for fat storage within adipose tissue are the adipocytes. Adipocytes contain large lipid droplets that encompass more than 95% of the entire body of the cell. The lipid droplet is used as a storage vessel for triglycerides. As the majority of the cell is dedicated to the storage of lipids, this led to the classic idea that adipose tissue's sole responsibility was as a passive fat storage system. Over the past few decades, however, this viewpoint has undergone a dramatic shift. Adipose tissue is now considered to be a complex endocrine and immune organ (Grant & Dixit,

2015; Booth *et al*., 2016). The discovery of leptin in the early 1990's by Zhang and colleagues (1994) led to an expansion in the understanding of the various roles that adipose tissue plays. Firstly, leptin was acknowledged as a regulator of body weight through the control of appetite and energy expenditure (Friedman & Halaas, 1998). It was then demonstrated to have an important relationship with the innate and adaptive immune system (Sanchez-Margalet *et al*., 2003). Leptin's roles include promoting the production of IL-1, IL-6, IL-12, and TNF-α, and stimulating the innate immune response. Further to this, leptin is able to activate neutrophil chemotaxis, which in turn stimulates the production of reactive oxygen species (ROS), promoting the activation of phagocytosis by macrophages and their secretions (Zhao *et al*., 2003), demonstrating that leptin has the capability to act as a pro-inflammatory cytokine.

Adipose tissue has the ability to secrete a variety of adipokines as well as pro- (e.g. TNF-α, IL-1β) and anti-inflammatory (e.g. IL -10) cytokines (see Table 3). These proteins are capable of modifying insulin sensitivity both locally within adipose tissue and systemically (Grant & Dixit, 2015). Makki and co-workers (2013) discussed that adipose tissue of lean individuals favours the production of anti-inflammatory adipokines such as adiponectin, transforming growth factor beta (TGFβ), IL-10, IL-4, IL-13, and apelin, whereas, in contrast, the adipose tissue in obese individuals mainly releases pro-inflammatory adipokines including TNF-α, IL-6, angiotensin II, and plasminogen activator inhibitor 1 (Ouchi *et al.*, 2011). Increased adiposity is therefore associated with increased levels of circulatory pro-inflammatory cytokines and acute-phase proteins (Pi-Sunyer, 2002; Caballero, 2003; Berg & Scherer, 2005).

Consequently, obesity has been proposed as a state of low-grade chronic inflammation (Festa *et al*., 2001; Gregor & Hotamisligil 2011). Fundamental indicators are, however, not observed within this form of inflammation; redness, swelling, heat and pain are not present (Medzhitov, 2008). Instead, the inflammation in obesity is of a different nature; it is slow and unspecific, unlike normal inflammation that is rapid and focused to a site of injury or infection, which is normally neutralised and resolved (Hotamisligil, 2006). The use of circulating values of inflammatory cytokines can be useful to detect adipose deregulations and potential onset of metabolic disorders.

Table 1.3. Cytokines and their link to inflammation and obesity

Cytokine	Effect in obesity	References
Pro- inflammatory		
CRP	Elevated inflammation	Festa *et al*., 2001; Park *et al*., 2005
TNFα	Increased inflammation and increased glycaemic deterioration	Bulló *et al*., 2003; Park *et al*., 2005; Ouchi *et al*., 2011; Schmidt *et al*., 2015
IL-6	Obesity	Park *et al*., 2005; Ouchi *et al*., 2011
IL-1β	Increased glycaemic deterioration	Dalmas *et al*., 2014
Anti-inflammatory		
IL-4	Pro – M2 – properties	Schmidt *et al*., 2015
IL-10	Reduces T_{reg} activity	Schmidt *et al*., 2015

CRP: C-reactive protein; TNFα: Tumor necrosis factor alpha; IL-6: Interleukin-6; IL-1β: Interleukin-1 Beta; IL-4: Interleukin-4; IL-10: Interleukin-10

1.4. Weight loss interventions

1.4.1. Different approaches to weight loss

Weight loss has been associated with reductions in the risk of morbidity and mortality (Poobalan *et al*., 2007). There are various forms of therapeutic approaches to weight loss; these include pharmaceutical, surgical, behavioural changes, and dietary supplements (Avenell *et al*., 2004, Pories, 2008; Caveny *et al*., 2011)

Pharmaceutical options have been shown to have lower uptake as a method of obesity treatment. Kushner (2014) discussed that pharmaceuticals are shrouded in concerns over safety. Drugs previously developed for weight loss have been removed from the marketplace due to undesired side effects. One such example would be amphetamines which were released in the 1930's as appetite suppressors/anorexiants. The use of amphetamines carried the risk of addiction along with various side effects, such as euphoria, increased blood pressure, pulmonary hypertension, cardiac valvulopathy and even depending on dose, symptoms indicative of paranoid schizophrenia (Carney, 1988; Silverstone, 1992; Abenhaim *et al*., 1996; Jick, 2000). Side effects such as these led to a reduction in peoples' willingness to use pharmaceutical methods. Further development of these drugs allowed the desired anorectic effect without the stimulatory properties and potential addictiveness (as reviewed by Kushner, 2014). The most common of these appetite suppressors are the five compounds phentermine, benzphetamine, phendimetrazine, diethylpropion and mazindol. Mazindol was removed from the United Kingdom market in 1972 for links to risk of cardiac valvulopthay, whereas benzphetamine, phendimetrazine, diethylpropion and phentermine are still currently prescription based

drugs approved for a 12-week basis (as reviewed by Caveny *et al*., 2011). Another form of pharmaceutical approach is to use gastrointestinal fat blockers; a drug used in this approach is Orlistat. This drug is a synthetic hydrogenated derivative of the naturally occurring lipase inhibitor lipostatin. This is an over-the-counter gastrointestinal fat blocker aimed as an inhibitor of pancreatic, gastric and carboxylester lipases and phospholipase A2, which are used in the hydrolysis of dietary fat (as reviewed by Kushner, 2014). Pancreatic lipase is a triglyceride digestion enzyme that is located in the small intestine. The lipase is secreted from the pancreas and is responsible for the hydrolysis of triglycerides into glycerol and fatty acids (Lowe, 1994). Therefore, combatting obesity with inhibitors of this enzyme, which in turn supresses the digestion of triglycerides has been suggested as a major approach in the prevention and treatment of obesity (Cha *et al*., 2012). Orlistat, however, carries a list of side effects, principally diarrhoea, faecal incontinence, oily spotting, flatulence, bloating and dyspepsia (Padwal *et al*., 2004; Ioannides-Demos *et al*., 2006).

Bariatric surgery can be considered one of the most effective treatments for obesity (Pories, 2008). It has high success rate with large improvements to patients' weight and reduction in metabolic comorbidities, and is generally used in cases of morbid obesity (BMI≥40 kg/m^2) (Picot *et al*., 2009; Colquitt *et al*., 2014). However, it has its downsides, too. It is a major surgical intervention that carries significant risk of morbidity and perioperative mortality (Colquitt *et al*., 2013); the risks of surgery include poor myocardial reserve, significant chronic obstructive airways disease or respiratory dysfunction, non-compliance of medical treatment, and infection (Colquitt *et al*., 2014). This increased risk leads to many individuals not meeting the eligibility criteria for the surgical intervention (Colquitt *et al*., 2009). Bariatric surgery also includes long-term after surgery care, including supplementation with vitamins and iron. Finally, it can also present high failure rates in the long term (te Riele *et al*., 2010). (te Riele *et al.,* 2010; as reviewed by Puzziferri *et al*., 2014). This may be due to the individual's desire to eat unhealthy foods not being addressed or physiological issues being present (Sala *et al*., 2017).

It is also possible to combat the negative impact of obesity through changes in behaviour such as eating patterns and physical activity (Avenell *et al*., 2004). This method of weight loss is a common and widespread treatment. Wardle and colleagues (2000) found that within the United Kingdom, 28% of adults were reported to have been actively attempting to lose weight using behavioural changes. In addition, Nicklas and colleagues (2012) conducted a study on a population of obese individuals from the United States that demonstrated that 63% of the participants had attempted to lose weight within the previous year using behavioural change treatment. This method has limitations, though. Nicklas

and co-workers (2012) reported that success rates in self-guided behavioural changes to induce weight loss were limited over a long period. Within the study, 40% of participants had succeeded in losing ≥5% of their initial weight and 20% had succeeded in losing ≥10% (Nicklas *et al*., 2012). In line with these findings, Jeffery and colleagues' (2000) and the National Institutes of Health, National Heart, Lung, and Blood Institute's report (1998) showed that the challenge of the treatment of obesity is the long-term maintenance of the weight loss. The findings from these studies showed that within first year post treatment the majority of individuals displayed significant weight gain, ranging from a third to half the weight they had previously lost (National Institutes of Health, National Heart, Lung, and Blood Institute's report, 1998; Jeffery *et al*., 2000). Other studies found that only 20% of the participants who lost 10% of their initial body weight maintained this loss for a year post treatment (Wing & Hill, 2001). In contrast, Thomas and co-workers (2014) found that 87% of participants in the National Weight Control Registry were still maintaining at least a 10% weight loss after 10 years. Factors that contributed to weight regain, according to this study, were decreased leisure physical activity, dietary restraint, and frequency of self-weighing (Thomas *et al*., 2014).

1.4.2. Plant polyphenols as nutritional aids in the treatment of obesity

Various studies have presented the vast range of potential health benefits of natural products in the treatment of obesity (McDougall *et al*., 2008; Yun, 2010; della Garza *et al*., 2011). Example of this are bioactive compounds that act as pancreatic lipase inhibitors, including catechins, tannins, isoflavoniods, triterpene saponins or theasaponins (Han *et al*., 2001; Yoshikawa *et al*., 2002; Naksi *et al*., 2005; Karu *et al*., 2007; Guo *et al*., 2009; Lee *et al*., 2010). These chemicals fall under the term polyphenols. The vast array of polyphenols are classified based on structure, the phenolic hydroxyl groups being the common link between them. Variations in the primary aromatic rings, oxidation status, and functional groups delineate the individual polyphenols. Four main classifications of polyphenols exist in the majority of diets: flavonoids, lignans, phenolic acids, and stilbenes (Garcia-Villalba *et al*., 2010). Polyphenols have been suggested to have some potential in reducing the onset of obesity via the inhibition of enzymes involved in the metabolism of fat (Yoshikawa *et al*., 2002). In addition, polyphenol extracts have the ability to reduce glucose, triglycerides, low-density lipoprotein (LDL) cholesterol and total cholesterol levels in the blood, increase energy expenditure and reduce body weight and adiposity (Terra *et al*., 2009; Garcia-Lafuete *et al*., 2009). Various foods contain polyphenol; fruits, vegetables, nuts, seeds, cocoa, coffee, wine, and chocolate are common sources within the diet (Kyle *et al*., 2006; reviewed by Motilva *et al*., 2013).

Green tea contains an abundance of bioactive substances, including polysaccharides, caffeine and polyphenols, primarily catechins (Friedrich *et al*., 2012). When green tea extracts were administered to young men, it was observed that they greatly increased energy expenditure and fat oxidation (Dullo *et al*., 1999; reviewed by Xu *et al*., 2014). Since the publication of this research, various clinical trials have shown the effectiveness of tea preparations on combating obesity through the increase of energy expenditure; fat oxidation, weight loss, and weight maintenance (see Table 1.4.). A study by Nagao and co-workers (2005) demonstrated that there was a significant reduction in body weight, BMI, waist circumference and body fat mass in male participants who took a green tea extract compared to a control group (see Table 1.4). Nagao and colleagues (2005) presented that the decrease in measurement from baseline to post treatment in those who took the active compounds was significantly greater than the placebo group (−3.4 ± 0.5 cm and −1.6 ± 0.4 cm, respectively). The caffeine content was adjusted in both groups, so that the authors were able to attribute these effects to the polyphenols present in the green tea extract: the catechins (Nagao *et al*., 2005). Further to this, Suliburska and colleagues (2012) showed that when their participants had their diets supplemented with green tea extract a significant decrease in BMI and waist circumference compared to placebo was observed even when the dose of catechins was reduced to a third of that in the study conducted by Nagao and co-workers (2005) (see Table 1.4.). Interestingly, Maki and co-workers (2009) observed that the benefits obtained from physical exercise were also increased in individuals consuming catechins (~625 mg) compared to a control group (Maki *et al*., 2009). They suggested that consumption of catechins could enhance the effects of exercise in weight loss (see Table 1.4.).

Another potential health benefit of polyphenols is the claim that they are effective in reducing inflammation both in general, and that related to obesity (Chiva-Blanch *et al*., 2014; Martínez-González *et al*., 2015). Various epidemiological studies have suggested that populations who have polyphenol-rich diets tend to have decreased prevalence of inflammatory disease (Yoon & Baek, 2005). It should be noted that there may be cofounding present; the effects of the polyphenol-rich diets could be attributed to other factors such as olive (Jurado-Ruiz *et al*., 2017) and fish oils (de Boer *et al*., 2016) in Mediterranean diets, or high fibre (Jiao *et al*., 2015). Mediterranean style diets are shown to have beneficial effects on inflammatory disease due to the significant amounts of bioactive components, including polyphenols (Siriwardhana *et al*., 2013). Wang and co-workers (2014) discuss in their review that several cell, animal and human studies have demonstrated that dietary bioactive

compounds act as anti-oxidants and anti-inflammatory agents, increasing thermogenesis and energy expenditure while decreasing inflammation and oxidative stress (Kalupahana & Moustaid-Moussa, 2012). In addition to this, it has been reported that polyphenols have the ability to suppress the growth of adipose tissue through antiangiogenic activity and the modulation of adipose tissue metabolism, suggesting they could be useful therapeutic modulators of obesity (Lin & Lin-Shiau, 2006).

There is controversy in the literature regarding the effects of treatments with polyphenols on the most common metabolic syndrome biomarkers. For example, studies using supplementation with green tea (Basu *et al*., 2011), cranberries (Broncel *et al*., 2010), aronia (Stull *et al*., 2010), blueberries (Egert *et al*., 2009), quercetin and resveratrol (Van der Made *et al*., 2015) showed no significant changes in the production of C-reactive protein (CRP). In contrast, studies using a citrus-based juice and hesperidin supplementation demonstrated a significant reduction in CRP and improvement in endothelial function in patients diagnosed with metabolic syndrome (Rizza *et al*., 2011). In this line, trials have shown that diets high in fruit and vegetables are associated with a decrease in IL-6 and IL-1 in obese individuals (Yeon *et al*., 2012) and improved vascular heath in subjects with cardiovascular disease (Macready *et al*., 2014). An increase in dietary consumption of vegetables has been shown to decrease the concentrations of IL-6 but not of other inflammation markers (Navarro *et al*., 2014). Conversely, there have been trials that have not found any change in inflammation and oxidative state between subjects in dietary intervention or control groups (Almendingen *et al*., 2005; Freese *et al*., 2004).

Green tea's abundance of polyphenols has been shown to reduce inflammation markers such as IL-6, IL-1, CRP, monocyte chemoattractant protein-1 (MCP-1), and granulocyte-macrophage colony-stimulating factor (GM-CSF) in animal studies (Chen *et al*., 2011; Lu *et al*., 2012). Green tea supplementation has led to decreased serum levels of amyloid alpha (SSA) (Kovacs *et al*., 2004), TNF-α (Bogdanski *et al*., 2012), CRP (Basu *et al*., 2011), and leptin (Wang *et al*., 2008). In contrast, various studies have found that diets supplemented with green tea had no effect on levels of inflammatory markers such as CRP, IL-6, IL-1, vascular cell adhesion molecule-1 (VCAM-1), soluble intercellular adhesion molecule-1 (sICAM-1), adiponectin, and leptin (Basu *et al*., 2011).

Various studies have demonstrated the potential of polyphenols as treatment for reducing obesity and obesity-driven inflammation. However, this plethora of studies presents various conflicting findings, clearly indicating the need for further research into their effectiveness.

Table 1.4. Polyphenols and their effect upon obesity and metabolic syndrome

Polyphenol	Effect	Author
Flavonoids	Significant reduction in body weight, BMI, waist circumference and body fat percentage in males with a doses of green tea (oolong tea containing 690mg catechins, compared to control group who got oolong tea containing 22mg catechins)	Nagao *et al*., 2005
	Significant decrease in BMI and waist circumference compared to placebo was observed ($p = 0.03$ and $p = 0.04$; respectively), even when the dose of catechins (379mg of green tea extract, versus a placebo) was reduced to a third of that in the study conducted by Nagao and co-workers (2005).	Sulibruska *et al*., 2012
	Percentage in total abdominal fat area [−7.7 (−11.7, −3.8) vs. −0.3 (−4.4, 3.9); $P = 0.013$], subcutaneous abdominal fat area [−6.2 (−10.2, −2.2) vs. 0.8 (−3.3, 4.9); $P = 0.019$], and fasting serum triglycerides (TG) [−11.2 (−18.8, −3.6) vs. 1.9 (−5.9, 9.7); $P = 0.023$] were greater in the catechin group. They suggested that consumption of catechins could enhance the effects of exercise in weight loss.	Maki *et al*., 2009
	Green tea containing caffeine (104mg/d) and catechins (573mg/d). Subjects lost 6.4 (±1.9) kg or 7.5 (±2.2) % of their original body weight ($P < 0.001$)	Kovacs *et al*., 2004
Stilbenes	Numerous pathways are associated with fat metabolism from stilbenes such as adiposegenesis, lipolysis, and thermogenesis.	Chou *et al*., 2018
Lignans	Improved glycaemic control, at least in part, by enhancing insulin signalling and sensitivity in diet-induced obese mice	Wang *et al*., 2015
Phenolic acids	Improved β-cell regeneration, insulin secretion, and lipid profiles	Lartha and Daisy, 2011
	Glucose uptake through translocation and activation of glucose transport (GLUT)4 in phosphatidylinositol-3 kinase (PI3K)/p-Akt pathway	Gandi *et al*., 2014
Tannins	Lipase inhibitors and lipolytic	Yoshikawa *et al*., 2002

1.4.3. Predictors of responsiveness to weight loss interventions

A major challenge related to the success of a weight loss intervention program is the identification of participants who are not responsive to that specific method (Teixeir *et al*., 2004; Elfhag & Rossner, 2005; Teixera *et al*., 2010; Bacon & Aphramor, 2011). Successfulness of weight loss is not identical in every person and great variability is observed within the same treatment. Interesting ideas about tailored approaches towards weight loss programs have been proposed as a result of analysis of the interaction between genes, dietary habits and behavioural factors (Ordovas, 2008).

A major contribution to the difference in success of the weight loss intervention programs has been attributed to the compliance of the participants (Wright *et al*., 2010). Non-compliance of participants has presented variability within weight loss interventions based on calorie restrictions (Foster *et al.*, 1992; Reicke *et al*., 2010). Foster and co-workers (1992) and Reicke and co-workers (2010) both found similar weight loss when they compared diets with calorie intakes of 400kcal/day and 800kcal/day. This is most likely because of participants' difference in compliance with the calorie-restricted diets. In these studies, between 30-50% of the participants had dropped out of the treatments within 3 to 6 months. Compliance is seriously affected in weight loss intervention programmes in free-living situations, and over extended periods of time (Crichton *et al*., 2012; Desroches *et al*., 2013).

Stroeve and co-workers (2016) observed that a relationship existed between weight loss success and the participants' metabolic function at baseline, and that this relationship was stronger in those participants who were morbidly obese. This stronger relationship may be due to the increased energy needs observed in morbidly obese individuals (Stroeve *et al*., 2016). Other factors shown to influence weight loss interventions' success include levels of physical activity (Jakicic, 2009; Delany *et al*., 2014), resting metabolic rate, and resting respiratory exchange ratio (Zurlo *et al*., 1990; Bray *et al*., 2012; Ellis *et al*., 2012). Other studies showed increased weight loss in those subjects with higher baseline bodyweight values (Hansen *et al.*, 2001; Greenberg *et al*., 2009; Finkler *et al*., 2012). This indicates those individuals with increased body mass may more easily reach an energy deficit.

Current literature within this field is limited in many areas such as biochemical, endocrine and immunological markers. The present study attempted to identify potential predictors of weight loss through the analysis of participants' anthropometric measurements, and biochemical, endocrine and immunological markers at baseline.

1. Hypotheses and Objectives

The purpose of this study was to investigate the relationship between anthropometric, metabolic, endocrine and immunological factors in a group of overweight/obese subjects before and after participating in a weight loss intervention programme, based on the consumption of two beverages consisting of green tea, green coffee and apple extracts.

Secondly, this study attempted to identify potential predictors of responsiveness to the dietary intervention.

2.1. The main objectives

1) To explore the relationships between indices of obesity (BMI, body fat %, WC, WHR, and WHeR) and early developmental factors, dietary habits, markers of metabolic function, markers of inflammation and counts of circulating immune cells at baseline in the participants.
2) To investigate the effect of a dietary polyphenol-based weight loss intervention on anthropometric measurements, body composition and the biochemical, endocrine and immune parameters studied.
3) To assess the individual responsiveness to the weight loss intervention program, and determine if and what factors may be predictors of responsiveness.

2.2. Hypotheses

1) Post-treatment anthropometry and body composition values will be reduced compared to baseline values.
2) Biochemical, endocrine and immunological levels will be improved as a result of the dietary intervention.
3) Compliance with treatment, caffeine consumption, and indices of obesity (BMI, body fat %, WC, WHR, WHeR,) will have the ability to predict responsiveness to this weight loss intervention.

2. Methods

3.1. The PRONAOS Project

The database used within this study was sourced by one of the subprojects of the PRONAOS project. PRONAOS was a specific project within the NAOS strategy (*Estrategia para la Nutrición, Actividad Física y Prevención de la Obesidad,* in Spanish: Nutrition, Physical Activity and Obesity Prevention Strategy) from the Spanish Government.). PRONAOS was an initiative of the former (2008) Spanish Ministry of Science and Innovation, included in the CENIT (*Consorcios Estratégicos Nacionales en Investigación Técnica*) programme, with the objective to encourage the cooperation between public and private sectors to improve R+D+I in Spain. A consortium was formed between industry and research institutions and universities with the idea to contribute to the scientific development of new food products in order to help reduce the obesity epidemic.

The subproject that generated the data for this study was held between the food group DAMM and the Immunonutrition Research Group from the Institute of Food Science, Technology and Nutrition of the Spanish National Research Council (ICTAN-CSIC).

My role within this study consisted on data processing and analysis, and the interpretation of the results from this subproject.

3.2 Weight loss intervention

3.2.1. Participants and experimental design

Participants were male and female adults (≥18 years of age; 38 women, 53 men) with a BMI of ≥ 24.5 kg/m^2, but otherwise healthy. They were recruited through posters, leaflets, Facebook and email. Written informed consent was obtained from all participants. The study protocol was reviewed and approved by the CSIC (Spanish National Research Council) Bioethics Committee and the Ethics Committee of the University Hospital “Puerta de Hierro” (Madrid, Spain) (see Annex 1 & 2).

The experiment consisted on a randomized, double-blind, cross-over intervention. Participants were randomly assigned to one of four groups: two groups received an active beverage and two received a placebo. The active drinks had differing concentrations of bioactive ingredients that may be prospective tools in the reduction and management of body weight. These ingredients are mainly polyphenols present in green tea, green coffee, yerba mate and apple, plus L-carnitine. Drinks were tested under a double-blind procedure. Participants had to drink 1 litre of the corresponding beverage

every day for 8 weeks. After that time, the participants underwent a washout period of minimum 4 weeks before repeating the protocol with a different treatment (placebo for those who had drunk a functional drink and *vice versa*) (Figure 3.1). Drinks were provided in powdered form, in specially designed plastic caps that allowed preservation of the formula and released the content into a 0.5 l plastic bottle containing water after being pressed.

The participants were monitored six times during the whole intervention: at the beginning of each phase, at week 4 in each phase, and at the end of each phase. Of the 91 initial participants, 79 completed both phases of the intervention.

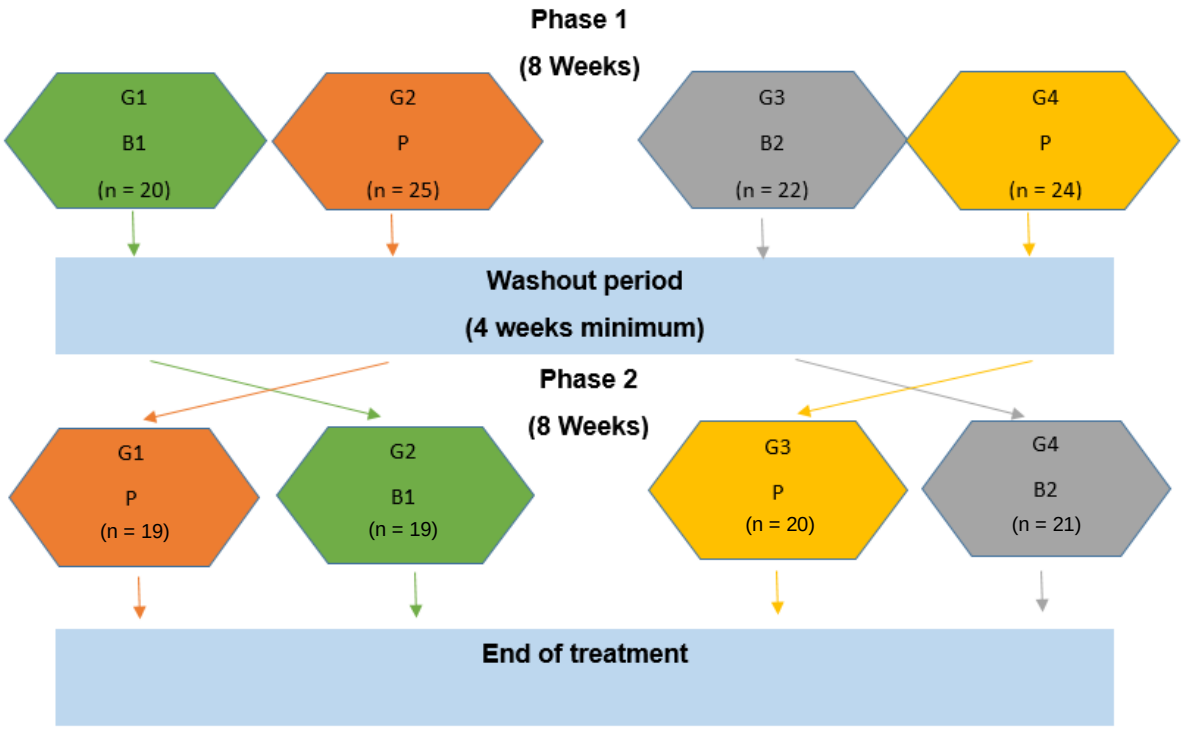

Figure 3.1. Experimental design of the weight loss intervention. G: group; B: active; P: placebo.

3.2.2. Personal history and dietary habits

Various information was gathered from the participants using a questionnaire. These data included: gender, age, country of birth, infant feeding style (breastfeeding or formula, which was decided as method received for the first 4 months), and birth weight.

Meal frequency was recorded from 3-day dietary records that included 2-week days and 1 weekend/ festive day.

Caffeine consumption was estimated from a food frequency questionnaire, and the following were calculated: total caffeine (mg/d), caffeine from tea and coffee (mg/d), caffeine from caffeinated and energy drinks per day (mg/d).

Compliance with the treatment was recorded by requesting the participants to return any unused caps at the end of each phase, and calculated as the percentage of caps consumed in relation to caps provided.

3.2.3. Anthropometrics

Anthropometric measurements were conducted on each of the monitoring sessions. During the meeting, body weight and body fat % were determined with a bioimpedance scale (BC-545, Tanita, Tokyo, Japan), and waist circumference and hip circumference were measured with a flexible, inextensible tape; girth measurements were taken three times and the median values were chosen. From these measurements, the following indices were calculated: BMI (kg/m^2), waist-to-hip ratio (WHR) and waist-to-height ratio (WHeR).

3.2.4. Blood analysis

Fasting blood samples were collected between 8 and 9 am, in vacuum tubes (Vacuette España, S.A.). EDTA tubes were used to collect blood samples (3 ml) for the haemogram and lymphocyte subpopulations; in order to obtain serum, 10 ml blood samples were collected using dry gel tubes; 2 ml blood samples were collected in EDTA tubes and the protease inhibitor aprotinin was added at a concentration of 6.5 µg/ml blood (1 µM) for plasma measurements.

Aliquots of blood and serum were sent to an external laboratory (Sanilab Labco, Spain) by refrigerated courier for the analysis of:

- Complete haemogram: red blood cells ($x10^6 cells/mm^3$), haemoglobin (Hb) (g/dl), haematocrit (%), mean corpuscular volume (fl), mean corpuscular Hb (pg), mean corpuscular Hb concentration (g/dl), platelets ($x10^3/mm^3$), white blood cells ($x10^3/mm^3$), neutrophils ($x10^3/mm^3$), lymphocytes ($x10^3/mm^3$), monocytes ($x10^3/mm^3$), eosinophils ($x10^3/mm^3$), and basophils ($x10^3/mm^3$)
- Biochemistry, hormones and acute phase proteins: concentrations of glucose (mmol/L), non-esterified fatty acids (NEFA) (mmol/L), triglycerides (mmol/L), total cholesterol (mmol/L), very low-density lipoprotein cholesterol (VLDL-c) (mmol/L), low-density lipoprotein cholesterol (LDL-c) (mmol/L), high-density lipoprotein cholesterol (HDL-c)

(mmol/L), apolipoprotein A (apoA) (mmol/L), apolipoprotein B (apoB) (mmol/L), iron (mg/dL), ferritin (mg/dL), transferrin (mg/dL), ceruloplasmin (mg/dL) and C-reactive protein (CRP) (mg/dL).

Insulin resistance was estimated by the homeostatic model assessment (HOMA), calculated as:

$$HOMA = \frac{Glucose(\text{mg/dl})\ x\ Insulin(mU/l)}{405}$$

Also, blood aliquots (500 ml) were immediately processed in the Immunonutrition laboratory to determine the percentages of several lymphocyte subpopulations, according to their membrane markers: total leukocytes ($CD45^+$), T mature cells ($CD3^+$), T helper ($CD4^+$), T cytotoxic ($CD8^+$), B lymphocytes ($CD3^-CD19^+$), natural killer cells ($CD3^-CD16^+56$), memory cells ($CD3^+CD45RO^+$, $CD4^+CD45RO^+$ and $CD8^+CD45RO^+$) and naive cells ($CD3^+CD45RA^+$, $CD4^+CD45RA^+$ and $CD8^+CD45RA^+$). Blood aliquots were incubated for 30 minutes at room temperature in the dark with fluorochrome-conjugated monoclonal antibodies (BD Biosciences, San José, CA, USA). A quadruple immunostaining procedure was performed as follows: CD3/CD8/CD45/CD4, $CD3/CD16^+56$/CD45/CD19, CD45RA/CD45RO/CD4/CD3, and CD45RA/CD45RO/CD8/CD3. Once lysis of red blood cells was completed, flow cytometry (FACScan Plus Dual Laser, Becton Dickinson Sunnyvale, CA) was used to analyse the lymphocytes. The lympho-gate was defined on the forward and side scatter patterns of lymphocytes. The analysis protocol gated on lymphocytes stained with two of the fluorochromes and the selected population was then analysed with the two remaining colours, to obtain percentages of cell expressing the specific antigens.

For further analysis of hormones and cytokines levels plasma samples were stored at −80 °C. Briefly, levels of insulin, ghrelin (pg/ml), leptin (ng/ml), adiponectin (ng/ml), interleukin-1 beta (IL-1β) (pg/ml), interleukin-2 (IL-2) (pg/ml), interleukin-4 (IL-4) (pg/ml), interleukin-6 (IL-6) (pg/ml), interleukin-8 (IL-8) (pg/ml), interleukin-10 (IL-10) (pg/ml), granulocyte macrophage colony-stimulating factor (GM-CSF) (pg/ml), interferon gamma (IFN-γ) (ng/ml), tumour necrosis factor alpha (TNF-α) (pg/ml), vascular cell adhesion molecule 1 (VCAM-1) (ng/ml), intercellular adhesion molecule 1 (ICAM-1) (ng/ml) were measured with the Bio-Plex® system and Luminex® xMAP technology (Bio-Rad Laboratories, Inc., USA) using a high sensitivity kit (Bio-Rad Laboratories, Inc., USA) and flow

cytometer (Bio-Plex® 200 system, Bio-Rad Laboratories, Inc., USA). The same kit was used to analyse all the samples and in the same day to avoid inter-assay variation. Beads that have been dyed with fluorochromes, associated optics, and high-speed digital signal processor are used in this system to identify and detect up to 100 different types of molecules in each single well of a 96-well microplate. The color-coded beads are pre-coated with analyte-specific capture antibodies, which bind to the hormone/cytokine of interest. Then, biotinylated detection antibodies specific to the analytes of interest are added and form an antibody-antigen sandwich. Finally phycoerythrin-conjugated streptavidin is added, binding to the biotinylated detection antibodies. Dyed beads are read on the Bio-Plex® analyser. One laser classifies the bead and determines the analyte that is being detected while a second laser determines the magnitude of the phycoerythrin-derived signal, which is in direct proportion to the amount of molecule bound.

3.3. Work conducted as part of the MPhil investigation: statistical analysis

All analysis was performed with the statistical program IMB SPSS 23. Significance was set $P<0.05$. All variables were checked for normal distribution using Shapiro-Wilk test prior to statistical testing.

3.3.1. Description of the sample

A description of the participants (n=91 at baseline; 79 completed the intervention) of the project was conducted according to the variables measured during the intervention. These variables were compared between genders due to the impact gender has upon many of them. Three participants who had indicated that they had taken laxatives were excluded from anthropometric measurement and body composition analysis.

Participants were categorised according to BMI as obese (≥30kg/m^2) or overweight (≥25kg/m^2) (National Health Service Choices, 2016). Participants were classified as abdominally obese or not under three different criteria: waist (≥94 cm for males, ≥80 cm for females; National Institute for Health Care Excellence, 2014); WHR (≥1:0 males, ≥0.85 females; WHO, 1999); and WHeR (≥0.5 for males and females; Hsieh *et al*., 1995).

Differences between genders were tested using the Chi Squared test for infant feeding style and country of birth. For the rest of variables analysed, genders were compared by the independent-*t* test or Mann-Whitney test, according to normality.

Analysis of covariance (ANCOVA) (abdominal obesity marker×gender) was used to compare average values of the variables studied between abdominally obese and non-abdominally obese participants, according to WC, WHR and WHeR.

3.3.2. Relationships between obesity and studied parameters

The indices of obesity were tested against the participants' infant feeding style, controlling for gender. A two-way ANOVA test was used to compare the indices of obesity between those who received breast milk and those who received formula. Participants who received both breast milk and formula simultaneously were excluded from this analysis.

Partial Pearson correlations controlling for gender were used to analyse the relationships between markers of obesity, birth weight, caffeine consumption and all blood parameters studied.

Finally, participants classified as overweight (≥25kg/^{2}) or obese (≥30kg/m^2) were compared for their baseline anthropometry and blood parameters using an independent *t*-test or a Mann-Whitney test, according to normality.

3.3.3. Analysis of the effects of the treatments

Baseline and post-treatment values of the indices of obesity and blood parameters were compared within each treatment group, separately in male and female participants, using a paired *t*-test or a Wilcoxon test, depending upon normality. These variables were also analysed by two-way ANOVA, in order to compare between baseline and post treatment values, and between placebo and functional drinks at the same time, and to account for the interaction between these two factors. This analysis was conducted in males and females separately, as well as in the whole sample.

In addition, relative changes were calculated for each participant, and compared between the four drinks (the two functional treatments B1 and B2 and their respective placebos P1 and P2). Kruskal-Wallis and ANOVA tests were used for comparison, depending on the normality of the data.

3.3.4. Potential predictors of weight loss

In order to identify possible predictors of responsiveness to the intervention only the data from the functional drinks (B1 and B2) were used.

The relative change of the participants' body weight was calculated. Participants were then ranked from lowest negative change to the highest positive change. Subjects were put into three evenly sized categories: high responsiveness, with a loss of more than 1.5% of body weight; low responsiveness, with any increase in body weight; and the middle category, which included participants with a relative body weight loss between 1.49% and 0% such as maintainers.

Distribution of males and females within high and low responsiveness groups was compared by the Chi-squared; this was also done for the distribution of the functional drinks (B1 and B2).

A correlation analysis was run between the relative body weight change and compliance with the treatment, estimated caffeine consumption and the baseline values for anthropometry and all blood variables. The correlation analysis was run separately on male and female participants as gender appeared to exert a significant effect on these relationships.

In addition, compliance and baseline values for anthropometry and all blood variables were compared between low and high respondents with both male and females combined, as responsiveness was not significantly affected by gender.

Variables which presented significant associations with body weight change and were backed up by differences between high and low respondents were identified as a potential predictors of weight loss. A hierarchical multiple regression analysis was run with these potential predictors to produce a predictive model of responsiveness to the treatment for the whole sample, and for males and females separately.

4 Results

4.1 Description of the sample

4.1.1. Demographics, early developmental factors, caffeine consumption, and anthropometric characteristics

Participants were on average 27 years old, and 90% of them Spanish. The majority had been breastfed during the first 4 months of life, and birth weight ranged between 3-4 kg approx. No differences were found between genders (Table 4.1).

The variability in the caffeine consumption was high, ranging from no caffeine at all to the equivalent of 3-4 expresso cups (approx. 636 – 848 mg of caffeine) a day, with no significant differences observed between males and females (Table 4.2).

The average BMI of the participants corresponded to that of overweight (28.3 kg/m^2), with indication of abdominal fat deposition (WHeR>0.50) (Table 4.3). The proportion of participants defined as obese (BMI>30kg/m^2) was 27.7% (25.7% of the female subjects and 28.8% of the males, with no significant difference between genders) (Figure 4.1). When assessing the prevalence of abdominal obesity in the sample, there was great disparity concerning the three commonly used indicators (Table 4.4). When waist circumference and WHeR were used, they described the majority of females (>70%) as abdominally obese, in contrast to WHR, which displayed most females (>80%) as non-abdominally obese. Among male participants, waist circumference presented similar proportions of abdominally obese and non-abdominally obese, whereas WHR classified most men as non-abdominally obese (79%), and WHeR, in contrast, classified most male participants (77%) as abdominally obese.

Table 4.1. Demographics of the total sample and according to gender.

		All (N = 91)	Females (N = 38)	Males (N = 53)	*P*
Age (y)		27 (24-35)	27 (24-34)	27 (24-35)	0.684
Country of birth (% participants)	**Spain**	90.1	86.8	92.5	0.379
	Other	9.9	13.2	7.5	
Feeding style as an infant (%)	**Unknown**	2.2	0	3.8	0.426
	Breastfed	54.9	52.6	56.6	
	Formula	27.5	31.6	24.5	
	Both	15.4	15.8	15.1	
Birth weight (kg)		3.40 (3.00-3.60)	3.20 (2.90-3.50)	3.50 (3.13-3.80)	0.073

Data presented as median (Q1, Q3), or percentage of participants. Mann-Whitney test used to compare between genders; Chi squared test used to compare frequencies for country of birth and feeding style as an infant between genders. Feeding style defined by method received in the first 4 months of life. Significance set as *P*<0.05.

Table 4.2. Estimated caffeine consumption (mg/day) for total sample and according to gender.

	All (N = 91)	Females (N = 38)	Males (N = 53)	*P*
Total caffeine	110.4 ± 104.4	110.6 ± 96.7	110.2 ± 110.6	0.671
	0 - 424.8	1.9 – 332.8	0 – 424.8	
Caffeine from tea and coffee	75.8 ± 88.8	70.9 ± 83.9	79.4 ± 92.8	0.768
	0 - 296.1	1.9 – 296.1	0 – 286.9	
Caffeine from caffeinated and energy drinks	27.8 ± 45.4	25.1 ± 38.5	29.7 ± 50.0	0.658
	0 – 276.8	0 – 149.6	0 – 276.8	

Data presented as the mean (± SD) and range. Independent-*t* test was used to compare between genders, with significance set at P<0.05.

Table 4.3. Anthropometric and body composition measurements in total sample and according to gender.

	All (N = 91)	**Females (N = 38)**	**Males (N = 53)**	***P***
Weight (kg)	84.3 ± 12.4	**76.2 ± 8.6**	**90.1 ± 11.5**	**<0.001**
Height (cm)	171.0 ± 0.10	**162.0 ± 6.0**	**177.0 ± 7.0**	**<0.001**
Perception of BMI (kg/m^2)	27.9 (26.3 – 34.5)	27.75 (26.1 – 36.3)	28.0 (26.3 – 34.5)	0.525
BMI (kg/m^2)	28.3 (26.4 – 34.5)	28.7 (26.9 – 36.5)	28.0 (26.1 – 34.5)	0.726
Body fat (%)	29.2 ± 8.5	**37.9 ± 4.0**	**23.5 ± 5.0**	**<0.001**
Waist circumference (cm)	90.0 (84.5 – 107.5)	**85.0 (80.0 – 96.5)**	**92.0 (89.5 – 112.5)**	**<0.001**
Hip (cm)	109.1 ± 7.6	**111.9 ± 7.9**	**107.0 ± 6.7**	**0.002**
Waist-to-hip ratio	0.84 (0.78 – 0.97)	**0.77 (0.72 – 0.91)**	**0.87 (0.84 – 0.97)**	**<0.001**
Waist-to–height ratio	0.53 (0.50 – 0.63)	0.53 (0.49 – 0.63)	0.52 (0.50 – 0.63)	0.781

Data presented as the mean ± SD or median (Q1, Q3). Significant differences between genders at P<0.05 highlighted in bold. Mann-Whiney test used to compare perception of BMI, BMI, waist circumference, waist-to-hip-ratio, and waist-to-height-ratio between genders; Student's-*t* test used to compare weight, height and body fat between genders.

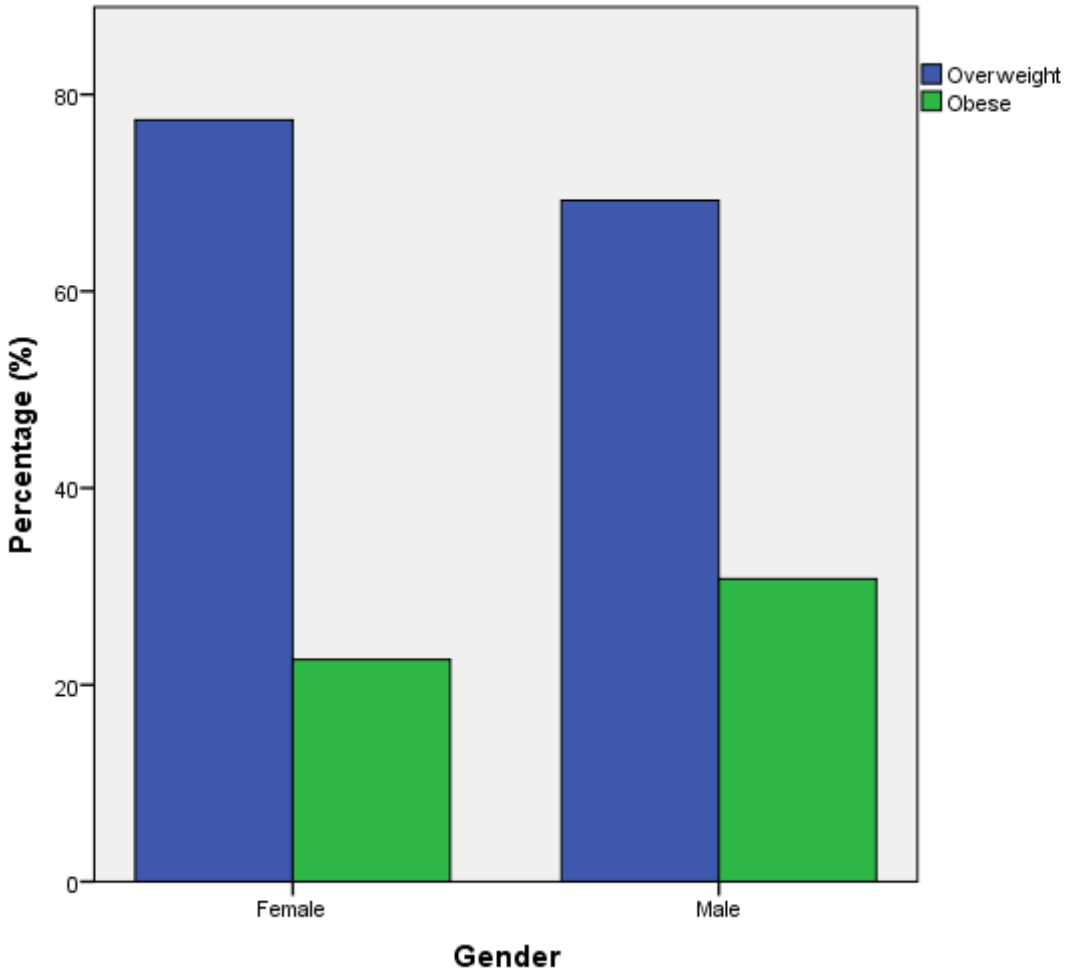

Figure 4.1. Classification of female and male participants according to body mass index as overweight (BMI ≥ 25 kg/m^2) or obese (BMI ≥ 30 kg/m^2). Chi square test was used to compare the proportions of the different categories between genders, significance set at P<0.05.

Table 4.4. Prevalence of abdominal obesity in the sample, according to three different markers.

		Females (n=35)	Males (n=53)
WC over threshold	**Yes**	74.3% (26)	43.4% (23)
	No	25.7% (9)	56.6% (30)
WHR over threshold	**Yes**	14.3% (5)	20.8% (11)
	No	85.7% (30)	79.2% (42)
WHeR over threshold	**Yes**	71.4% (25)	77.4% (37)
	No	28.6% (10)	22.6% (12)

Threshold for waist circumference (WC): 80 cm for females, 94 cm for males; for waist-to-hip ratio (WHR): 0.85 for females, 1.0 for males; for waist-to-height ratio (WHeR): 0.5 for both females and males.

4.1.2. Blood cells

Mean haemogram values for whole population fell within normal values for healthy adults. Female participants presented lower red blood cell counts, haemoglobin levels and haematocrit, higher platelet counts and transferrin, while male participants presented significantly higher levels of iron and ferritin (Table 4.5), while no differences were observed between genders for any of the leukocyte variables (Tables 4.6 and 4.7).

Table 4.5. Red blood cell series in total sample and according to gender

	All (N = 91)	**Females (N = 38)**	**Males (N = 53)**	**P**
RBC (x10^{6}cells/mm^3)	4.8 (4.5 – 5.5)	**4.5 (4.2 – 5.1)**	**5.0 (4.8 – 5.5)**	**<0.001**
Hb (g/dl)	14.1 ± 1.3	**13.1 ± 0.9**	**14.9 ± 0.9**	**<0.001**
Haematocrit (%)	43.0 ± 3.8	**40.0 ± 2.9**	**45.2 ± 2.7**	**<0.001**
MCV (fl)	89.7 ± 4.4	89.5 ± 4.8	89.9 ± 4.0	0.661
MCH (pg)	29.7 (28.6 – 31.8)	29.7 (27.6 – 37.7)	29.6 (28.8 – 31.8)	0.347
MCHC (g/dl)	32.9 ± 0.9	32.7 ± 0.9	33.0 ± 0 .9	0.058
Anisocytosis Index (%)	12.6 (12.0 – 13.2)	12.5 (11.8 – 13.4)	12.6 (12.1 – 13.2)	0.710
Platelets (x10^3/mm^3)	252 ± 51	**271 ± 53**	**249 ± 46**	**0.003**
Iron (mg/dl)	86.0 (59.0 – 160.0)	**76.5 (50.0 – 156.0)**	**94.0 (72.0 – 168.0)**	**0.022**
Ferritin (ng/dl)	54.0 (27.0 – 274.0)	**27.5 (13.0 – 75.0)**	**127.0 (58.0 – 292.0)**	**<0.001**
Transferrin (mg/dl)	250.1 ± 50.3	**278.5 ± 43.2**	**229.8 ± 45.2**	**<0.001**

Data presented as mean ± SD or median (Q1, Q3). Student's-*t* test used to compare haemoglobin (Hb), haematocrit, mean corpuscular volume (MCV), mean corpuscular Hb concentration (MCHC), and platelets between genders. Mann-Whitney test used to compare red blood cells (RBC) count and mean corpuscular Hb (MCH) between genders. Differences significant at $P < 0.05$.

Table 4.6. White blood cell counts in total sample and according to gender.

Cells (x10^3/mm^3)	**All (N = 91)**	**Females (N = 38)**	**Males (N =58)**	**P**
White blood cells	6.2 (5.2 – 9.4)	6.5 (5.2 – 9.6)	5.9 (5.0 – 9.2)	0.282
Neutrophils	3.2 (2.6 – 5.4)	3.3 (2.7 – 6.0)	3.1 (2.6 – 5.1)	0.173
Lymphocytes	2.1 (1.8 – 3.2)	2.1 (1.9 – 3.0)	2.1 (1.7 – 3.6)	0.974
Monocytes	0.5 (0.4 – 0.8)	0.5 (0.4 – 0.7)	0.5 (0.4 -0.8)	0.642
Eosinophils	0.2 (0.1 – 0.5)	0.1 (0.1 – 0.5)	0.2 (0.1 – 0.4)	0.386
Basophils	0.0 (0.0 – 0.1)	0.00 (0.0 – 0.1)	0.00 (0.0 -0.10)	0.688

Data presented as median (Q1, Q3). Mann-Whitney test used to compare white cell counts between genders. Significance set as $P<0.05$.

Table 4.7. Lymphocyte subset counts in total sample and according to gender.

Cells/mm^3	All (N = 91)	Females (N = 38)	Males (N =58)	P
CD3$^+$	1608.0 ± 479.8	1631.3 ± 421.1	1591.3 ± 521.1	0.697
CD3$^+$CD4$^+$	900.4 (720.0 – 1476.2)	922.5 (784.0 – 1476.2)	823.1 (658.8 – 1547.6)	0.145
CD3$^+$CD8$^+$	508.6 (382.6 – 994.5)	504.0 394.7 – 932.4)	508.6 (356.1 – 1006.7)	0.681
CD3$^+$CD19$^+$	237.2 ± 93.1	217.2 ± 73.3	251.5 ± 103.3	0.083
NKCD3$^-$ (NK)	237.3 (154.0 – 698.1)	229.2 (154.0 – 556.6)	239.0 (160.8 – 771.8)	0.486
CD4$^+$RA$^+$	353.42 (232.6 – 676.3)	373.2 (262.9 – 707.4)	315.2 (224.3 – 614.5)	0.102
CD4$^+$RO$^+$	476.1 (404.7 – 855.1)	526.1 (404.7 – 855.1)	455.3 (391.5 – 932.7)	0.421
CD8$^+$RA$^+$	297.2 (213.6 – 631.7)	301.9 (219.8 – 631.7)	288.1 (194.1 – 784.0)	0.551
CD8$^+$RO$^+$	211.7 (138.9 – 554.1)	188.6 (142.0 – 566.1)	214.8 (138.9 – 554.1)	0.543
NKCD3$^+$	70.6 (31.3 – 282.3)	71.2 (31.30 – 176.20)	69.2 (31.60 – 305.00)	0.655
CD4/CD8	1.80 (1.32 – 3.39)	1.87 (1.37 – 3.27)	1.84 (1.27 -3.62)	0.504
CD3/CD19	6.95 (5.39 – 15.09)	7.20 (5.68 – 18.46)	6.60 (5.00 – 12.63)	0.057

Data presented as the median (Q1, Q3) or mean ±SD. Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name. Mann-Whitney test used to compare between genders for CD4$^+$, CD8$^+$, NKCD3$^-$, CD4$^+$RA$^+$, CD4$^+$RO$^+$, CD8$^+$RA$^+$, CD8$^+$RO$^+$, NKCD3$^+$, CD4/CD8, and CD3/CD19; and Student's *t*-test used for CD3$^+$, and CD19$^+$. Significance set at P<0.05.

4.1.3. Biochemistry, hormones and cytokines

The average values of glucose, insulin and lipids fell within the normal range (Table 4.8). Female participants had slightly higher concentrations of glucose and insulin, which resulted in significantly higher values for the HOMA index. They also presented higher levels of total cholesterol, HDL-c and ApoA.

The concentrations of hormones, cytokines and adhesion molecules in the studied sample presented great variability. In addition, the levels of certain cytokines fell below the detection levels of the kit used for analysis. Female subjects presented significantly higher levels of leptin, ceruloplasmin, CRP, GM-CSF, and IFNγ. Male participants presented significantly higher levels of IL-1β (Table 4.9).

Table 4.8. Blood biochemistry and iron markers in the total sample and according to gender.

	All (N = 91)*	**Female (N = 38)***	**Male (N = 53)***	**P**
Glucose (mmol/L)	4.8 ± 0.7	5.0± 0.4	4.7 ± 0.8	0.076
Insulin (mU/l)	2.2 (1.5 – 12.8)	2.2 (1.57 – 4.4)	2.1 1.31 – 18.1)	0.717
HOMA	1.8 (0.0 – 10.9)	**2.8 (1.3 – 6.3)**	**0.5 (0.0 – 18.8)**	**0.006**
NEFA (mmol/L)	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.482
Triglycerides (mmol/L)	4.8 (3.2 – 12.3)	4.4 (3.1 – 9.6)	4.9 (3.2 – 13.6)	0.346
Total cholesterol (mmol/L)	9.3 (8.1 – 18.4)	**9.8 (8.7 – 13.4)**	**8.7 (7.7 – 11.9)**	**0.001**
VLDL-c (mmol/L)	0.9 (0.7 – 2.4)	0.9 (0.6 – 1.9)	1.0 (0.7 – 2.7)	0.348
LDL-c (mmol/L)	5.2 (4.6 – 8.1)	5.6 (5.0 – 8.8)	5.2 (4.6 – 7.9)	0.108
HDL-c (mmol/L)	2.6 ± 0.8	**3.2 ± 0.8**	**2.2 ± 0.6**	**<0.001**
APOA (mmol/L)	7.8 (7.2 – 11.1)	**8.1 (7.4 – 11.2)**	**7.4 (6.9 – 9.4)**	**0.008**
APOB (mmol/L)	4.4 (3.8 – 7.0)	4.4 (3.7 – 8.1)	4.6 (3.8 – 6.9)	0.693

Data presented as median (Q1, Q3) or mean ±SD. Student's-*t* test or Mann-Whitney test used, depending on normality. Significant differences between genders highlighted in bold, at P<0.05. HOMA: homeostasis model assessment; NEFA: non-esterified fatty acids; VLDL-c: very low-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; ApoA: apolipoprotein A; ApoOB: apolipoprotein B. *N values for insulin and HOMA: 84 (all), 36 (females), and 48 (males).

Table 4.9. Circulating levels of adipokines, acute phase proteins, cytokines, and adhesion molecules, in total sample and according to gender.

	N	All	N	Female	N	Male	P
Ghrelin (pg/ml)	90	274.7 (203.7 – 576.5)	38	251.1 (194.4 – 360.9)	52	283.0 (231.6 – 367.2)	0.356
Leptin (ng/ml)	**91**	4.84 (2.44 – 19.43)	**38**	**10.88 (7.86 – 16.36)**	**53**	**2.86 (1.43 – 4.24)**	**<0.001**
Adiponectin (ng/ml)	91	4726 (3285 – 13579)	38	5562 (3770 – 8755)	53	4601 (3285 – 6018)	0.055
Ceruloplasmin (mg/dl)	**91**	27.1 (23.7 – 43.4)	**38**	**29.2 (27.0 – 36.6)**	**53**	**25.0 (22.2 – 28.3)**	**<0.001**
CRP (mg/dl)	**91**	0.90 (0.21 – 11.82)	**38**	**1.31 (0.47 – 3.67)**	**53**	**0.52 (0.16 – 1.60)**	**0.005**
IL-1β (pg/ml)	**26**	0.89 (0.38 – 670.21)	**15**	**0.45 (0.17 – 1.10)**	**11**	**3.20 (0.85 – 9.13)**	**0.003**
IL-2 (pg/ml)	19	39.3 (10.6 – 8905.6)	1	66.6	18	30.9 (10.6 – 72.8)	0.632
IL-4 (pg/ml)	17	2.33 (0.68 – 4.84)	0	-	17	2.33 (0.68 – 4.84)	N/A
IL-6 (pg/ml)	91	13.1 (8.8 – 58.6)	38	14.0 (8.1 – 18.0)	53	12.9 (9.0 – 19.5)	0.968
IL-8 (pg/ml)	91	22.6 (16.3 – 174.9)	38	21.1 (16.3 – 33.8)	53	24.2 (16.0 – 49.0)	0.791
IL-10 (pg/ml)	33	11.2 (2.09 – 416.9)	13	4.4 (1.96 – 12.0)	20	14.7 (3.36 – 93.1)	0.094
GM-CSF (pg/ml)	**71**	291.7 (140.7 – 1779.2)	**25**	**714.8 (543.7 – 857.7)**	**46**	**226.4 (103.7 – 390.1)**	**<0.001**
IFNγ (ng/ml)	**38**	0.41 (1.75 – 144.1)	**6**	**2.23 (0.41 – 2.47**	**32**	**0.31 (0.10 – 0.77)**	**0.041**
TNF-α (pg/ml)	79	4.44 (1.75 – 144.05)	38	3.47 (1.75 – 10.17)	41	6.65 (2.69 – 27.39)	0.148
VCAM-1 (ng/ml)	91	108.9 (95.2 – 165.4)	38	108.6 (102.0 – 125.0)	53	109.5 (94.5 – 131.0)	0.859
ICAM-1 (ng/ml)	91	94.6 ± 25.78	38	93.7 ± 18.39	53	95.2 ± 30.7	0.783

Data presented as the median (Q1, Q3) or mean +SD. Student's-*t* test used to compare ICAM-1 between genders, and Mann-Whitney test used for all the other variables. Significance set at P< 0.05. CRP: C-reactive protein; GM-CSF: granulocyte macrophage colony-stimulating factor; IL: interleukin; IFNγ: interferon gamma; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1; ICAM1: intercellular adhesion molecule 1.

4.2. Relationships between indices of obesity and studied variables

4.2.1. Indices of obesity and early developmental factors

The average values in the indices of obesity were lower those participants who had been breastfed, the difference being significant for waist circumference for WHR, there was a significant interaction between feeding style and gender, with breastfed females but not males presenting lower values than the formula-fed counterparts (Table 4.10).

There were moderate significant relationships between birth weight and adult BMI. This was quadratic for females ($R^2 = 0.248$, $F_{(2, 28)} = 4.627$, $P = 0.018$) (Figure 4.2), suggesting a U-shaped association between birth weight and BMI, with the optimum range between approximately 3 – 3.5 kg. In males, the relationship was linear ($R^2 = 0.139$, $F_{(1, 49)} = 7.900$, $P = 0.007$) (Figure 4.3). Strong significant quadratic relationships were observed as well between birth weight and abdominal obesity indices; moderate in females (waist circumference: $R^2 = 0.358$, $F_{(2, 28)} = 7.802$, $P = 0.002$; WHeR: $R^2 = 0.311$, $F_{(2, 28)} = 7.006$, $P = 0.003$) (Figures 4.4 and 4.5), and moderate in males ($R^2 = 0.148$, $F_{(2,48)} = 4.181$, $P = 0.021$) (Figure 4.6).

Table 4.10. Body composition of sample according to gender and feeding habits as infants.

	Females (N =32)		**Males (N =43)**		***P* feeding**	***P* interaction**
	Breastfed	**Formula**	**Breastfed**	**Formula**		
N	20	12	30	13		
BMI (kg/m^2)	28.4 ± 3.0	30.2 ± 3.2	28.4 ± 3.1	29.2 ± 3.1	0.343	0.626
Body fat (%)	37.2 ± 3.3	39.5 ± 4.3	23.1 ± 5.3	24.1 ± 4.0	0.522	0.757
WC (cm)	**83.3 ± 5.6**	**89.8 ± 7.6**	**94.3 ± 9.4**	**96.1 ± 7.6**	**< 0.001**	0.160
WHR	**0.74 ± 0.06**	**0.82 ± 0.08**	**0.89 ± 0.06**	**0.88 ± 0.07**	0.234	**0.022**
WHeR	0.51 ± 0.04	0.57 ± 0.06	0.53 ± 0.05	0.53 ± 0.05	0.157	0.088

Data presented as mean ± SD. Two-way ANOVA test was used to analyse the effect of gender and feeding style. Significance set at P< 0.05. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ration; WHeR: waist-to-height ratio.

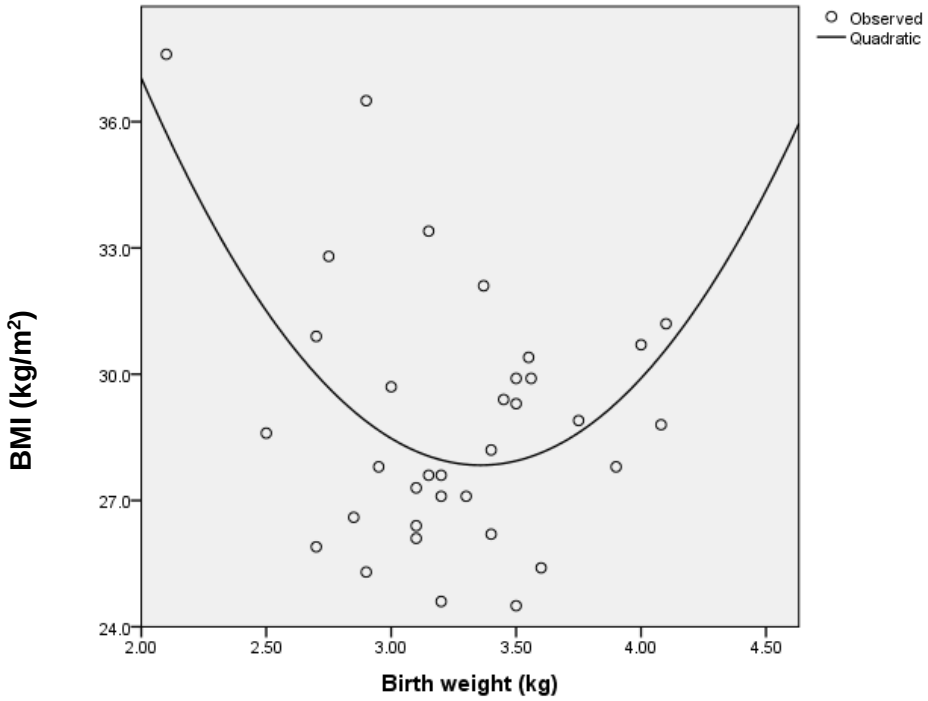

Figure 4.2. Association between birth weight (kg) and BMI (kg/m^2) in females (n=34, R^2 = 0.248, P = 0.018).

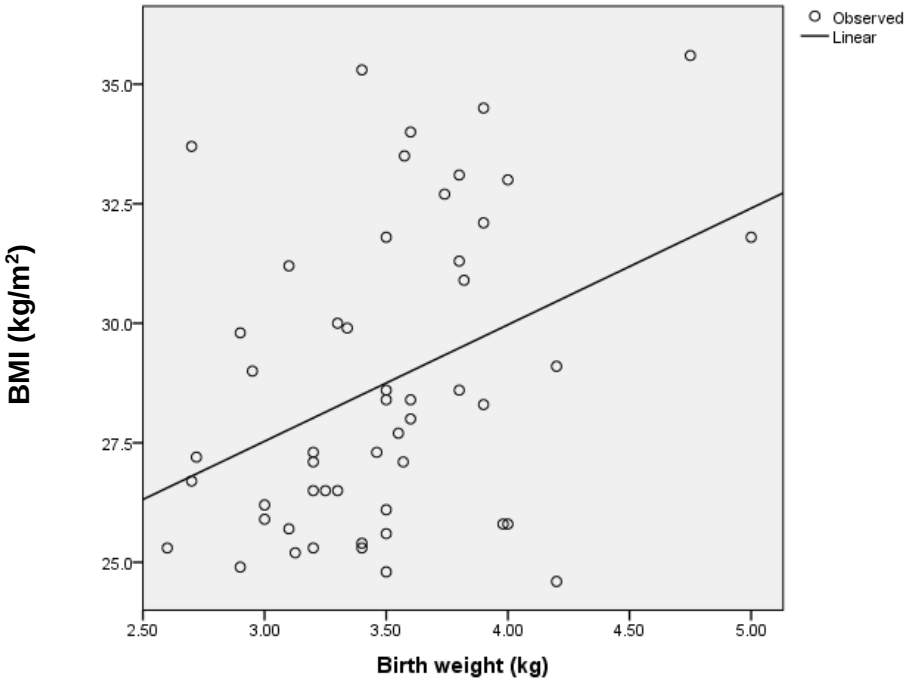

Figure 4.3. Association between birth weight (kg) and BMI (kg/m^2) in males (n=51, R^2 = 0.139, P = 0.007).

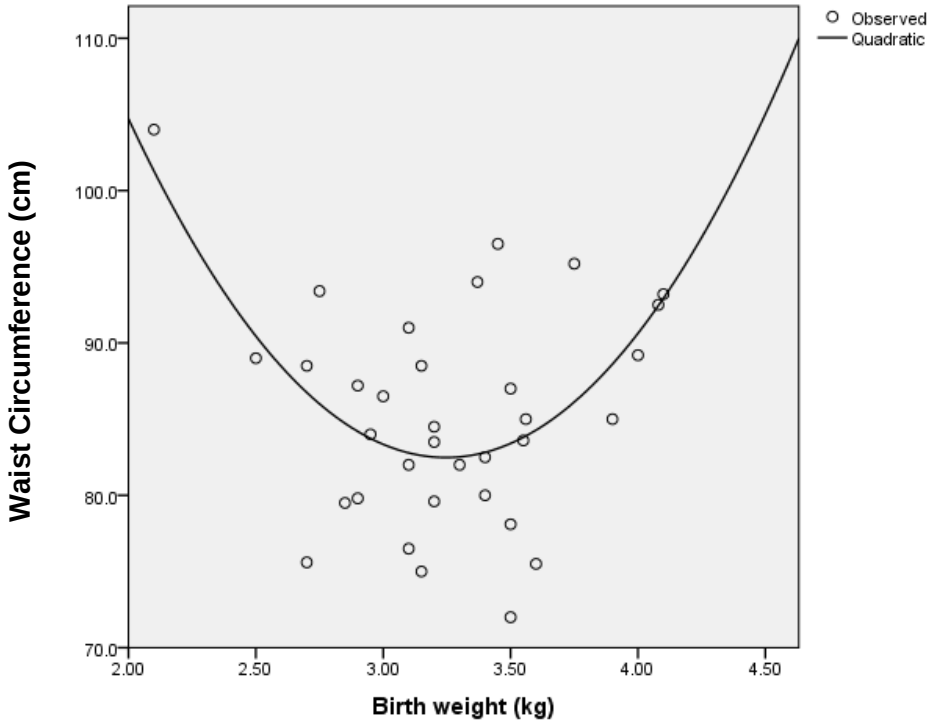

Figure 4.4. Association between birth weight (kg) and waist circumference (cm) in females (n=34, R^2 = 0.358, P = 0.002).

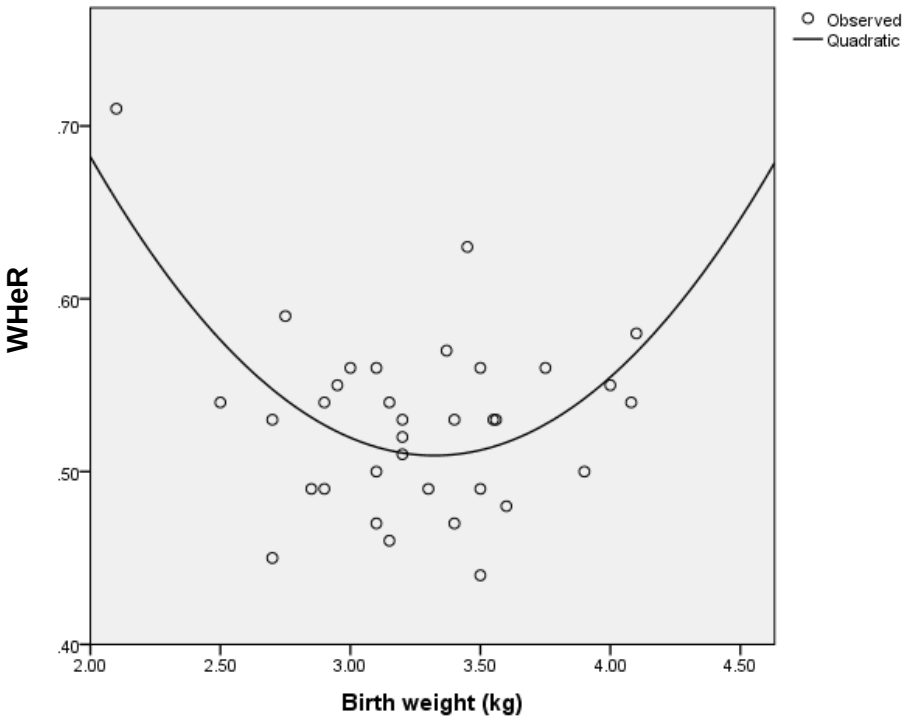

Figure 4.5. Association between birth weight (kg) and waist-to-height ratio (WHeR) in females (n=34, R^2 = 0.311, P = 0.003).

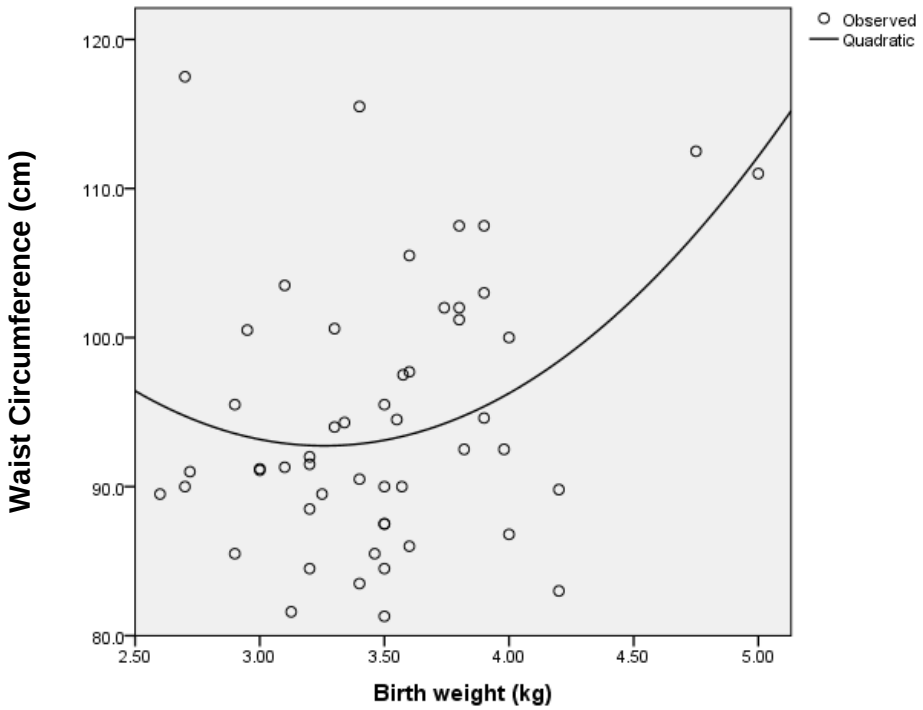

Figure 4.6. Association between birth weight (kg) and waist circumference (cm) in males (n=51, R^2 = 0.148, P = 0.021).

4.2.2. Indices of obesity, estimated caffeine consumption and dietary habits

None of the indices of obesity studied presented any significant correlations with total consumption of caffeine per day, caffeine from tea and coffee, or caffeine from caffeinated and energy drinks (Annex 9).

The majority of the participants presented a high frequency of meals per day; only 1 in 5 (24%) skipped breakfast always or sometimes, and 82% ate four or more times a day. Table 4.11 presents the average values for different indices of obesity in relation to the number and type of meals consumed by the participants. No significant difference was observed between individuals who consumed breakfast, mid-morning snack, lunch, afternoon snack and evening meals and those who did not. Similarly, there were no significant differences between participants who consumed a higher number of meals per day (4 or more) and those who had fewer meals (3 or fewer).

Table 4.11. Values of different indices of obesity according to meal distribution patterns.

		Female (N = 38)						Male (N= 53)					P_g	P_m	P_i
		N	BMI (kg/m^2)	Body Fat (%)	Waist (cm)	WHR	WHeR	N	BMI (kg/m^2)	Body Fat (%)	Waist (cm)	WHR	WHeR			
Breakfast	**Y**	28	28.8 ± 3.0	37.9 ± 4.4	86.4 ± 7.8	0.77 ± 0.07	0.53 ± 0.06	29	28.3 ± 2.8	23.2 ± 4.8	94.5 ± 9.3	0.89 ± 0.07	0.53 ± 0.05	0.278	0.386	0.478
	N/S	5	30.0 ± 3.7	38.6 ± 2.3	88.2 ± 6.2	0.78 ± 0.08	0.54 ± 0.01	13	28.4 ± 2.7	23.2 ± 4.9	93.5 ± 5.3	0.87 ± 0.06	0.52 ± 0.05			
Mid-Morning	**Y**	12	28.9 ± 3.3	38.2 ± 4.9	88.3 ± 7.4	0.77 ± 0.07	0.53 ± 0.07	12	28.1 ± 3.1	23.8 ± 5	92.6 ± 7.8	0.88 ± 0.09	0.53 ± 0.07	0.356	0.789	0.861
	N	5	28.8 ± 4.3	38.0 ± 4.4	83.6 ± 8.0	0.77 ± 0.08	0.52 ± 0.06	9	28.4 ± 2.7	22.2 ± 3.4	97.5 ± 9.6	0.87 ± 0.06	0.53 ± 0.04			
	S	16	29.1 ± 2.7	37.9 ± 3.7	87.0 ± 6.2	0.78 ± 0.08	0.53 ± 0.04	21	28.4 ± 2.8	23.3 ± 5.3	92.1 ± 3.2	0.89 ± 0.05	0.53 ± 0.04			
Lunch	**Y**	32	29.0 ± 3.1	37.9 ± 4.2	86.7 ± 7.7	0.77 ± 0.07	0.53 ± 0.05	39	28.5 ± 2.8	23.4 ± 4.8	94.7 ± 9.0	0.89 ± 0.06	0.53 ± 0.05	0.421	0.547	0.487
	N/S	1	28.8	40.2	87.0	0.82	0.54	3	26.2 ± 1.6	20.9 ± 4.6	89.8 ± 2.3	0.82 ± 0.04	0.49 ± 0.02			
Afternoon	**Y**	6	29.1 ± 2.6	38.0 ± 3.8	89.1 ± 6.6	0.76 ± 0.04	0.52 ± 0.04	13	26.9 ± 1.6	21.3 ± 4	93.8 ± 10.1	0.88 ± 0.05	0.52 ± 0.03	0.328	0.347	0.262
	N	12	28.9 ± 3.4	37.9 ± 4.0	83.8 ± 7.8	0.76 ± 0.09	0.52 ± 0.06	10	29.7 ± 2.6	25.2 ± 4.4	94.9 ± 7.9	0.91 ± 0.08	0.55 ± 0.06			
	S	15	29.1 ± 3.2	38.1 ± 4.7	78.5 ± 4.9	0.80 ± 0.06	0.55 ± 0.06	19	28.6 ± 3.1	23.4 ± 5.2	96.3 ± 11.5	0.87 ± 0.06	0.53 ± 0.05			
Evening	**Y**	31	29.0 ± 3.1	38.0 ± 4.1	87.5 ± 7.3	0.77 ± 0.07	0.53 ± 0.05	34	28.3 ± 2.7	23.0 ± 4.5	94.7 ± 9.1	0.89 ± 0.06	0.53 ± 0.05	0.341	0.784	0.698
	N/S	5	29.4 ± 2.3	39.4 ± 3.2	81.3 ± 8.0	0.79 ± 0.06	0.54 ± 0.01	8	28.2 ± 3.3	24.0 ± 6.0	91.8 ± 5.1	0.86 ± 0.08	0.53 ± 0.06			
≥4 meals/day		26	29.2 ± 2.8	38.5 ± 3.9	86.1 ± 6.7	0.78 ± 0.07	0.54 ± 0.05	33	28.3 ± 2.8	23.3 ± 5.0	94.8 ± 8.1	0.89 ± 0.06	0.53 ± 0.05	0.415	0.783	0.652
≤3 meals/day		5	28.3 ± 4.9	35.3 ± 5.1	83.2 ± 8.5	0.72 ± 0.06	0.49 ± 0.04	8	28.5 ± 3.2	22.9 ± 4.5	94.0 ± 9.3	0.87 ± 0.07	0.53 ± 0.05			

Data presented as the mean ± SD. Significance set at P<0.05, analysed by two-way ANOVA. P_g: Significance for gender effect; P_m: Significance for meal effect; P_i: Significance of the interaction. BMI: body mass index; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; Y: yes; N: no; S: sometimes; N/S: no/sometimes.

4.2.3. Indices of obesity and blood cells

Moderate but significant positive correlations were observed between all indices of obesity except WHR and red blood cell counts and platelets (Table 4.12). In this line, when overweight and obese participants were compared, the red blood cell count was significantly higher in the latter (4.75 ± 0.45 vs 4.96 ± 0.35 x10^6 cell/mm^3, respectively, P=0.035). In contrast, significant negative correlations were observed between BMI, waist circumference and WHeR and the size and haemoglobin concentration of red blood cells. Ferritin was positively correlated with BMI in the total sample (Table 4.12), until split according to gender, when females presented a negative trend (Figure 4.7).

All indices of obesity except body fat (%) presented weak but significant positive correlations with total white blood cell counts, however, when split according to gender only females presented a significant correlation (Figure 4.8). Positive correlations were also found between all indices of obesity and neutrophil counts (Table 4.13). Lymphocyte counts were inversely correlated with body fat (%) and positively with WHR. Monocytes showed significant positive correlations with BMI and WHeR (Table 4.13). BMI was also positively associated with memory T helper cells ($CD4^+RO^+$) while body fat (%) presented a significant negative correlation with T cytotoxic ($CD8^+$) memory/naïve ratio ($CD8^+RO^+/CD8^+RA^+$). Abdominal obesity (as WHR) was, however, positively correlated with T ($CD3^+$) T cytotoxic ($CD8^+$) cells, and memory T$CD8^+$cells (Table 4.14).

Table 4.12. Correlation coefficients between indices of obesity and red series and iron status.

	BMI	Body fat (%)	WC	WHR	WHeR
RBC	**0.307****	**0.260***	**0.266***	0.172	**0.341*****
Hb	0.152	0.129	0.91	-0.033	0.106
Haematocrit	0.147	0.111	0.101	0.032	0.144
MCHC	**-0.221***	-0.207	**-0.224***	-0.17	**-0.263***
MCV	**-0.221***	-0.194	**-0.236***	-0.254	**-0.315****
MCH	0.001	0.03	-0.029	-0.162	-0.106
Platelets	**0.270***	**0.251***	**0.253***	0.246	**0.384*****
Iron	-0.05	-0.153	-0.058	-0.113	-0.108
Ferritin	**0.329****	**0.326****	**0.373****	**0.246***	**0.350****
Transferrin	0.159	-0.043	0.189	0.141	0.168

Partial Pearson correlation adjusted for gender. Significance at *P<0.05, **P<0.01, and ***P<0.001. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; RBC: red blood cells; Hb: haemoglobin; MCHC: mean corpuscular Hb concentration; MCV: mean corpuscular volume; MCH: mean corpuscular Hb. N = 84.

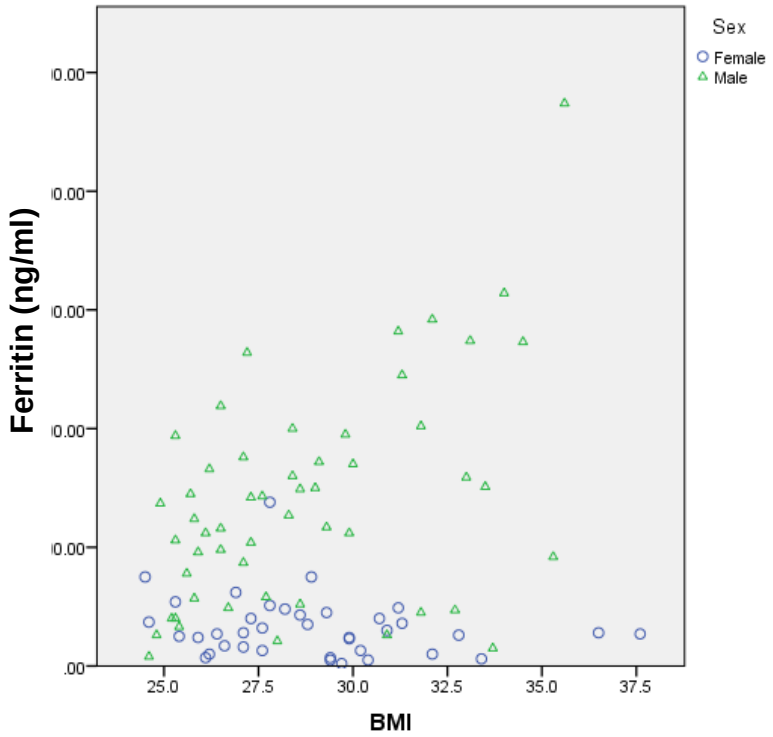

Figure 4.7. Association between ferritin (ng/ml) and BMI in females (n=34) and males (n = 52). Females: r = −0.188, P = 0.278; males: r = 0.472, P < 0.001.

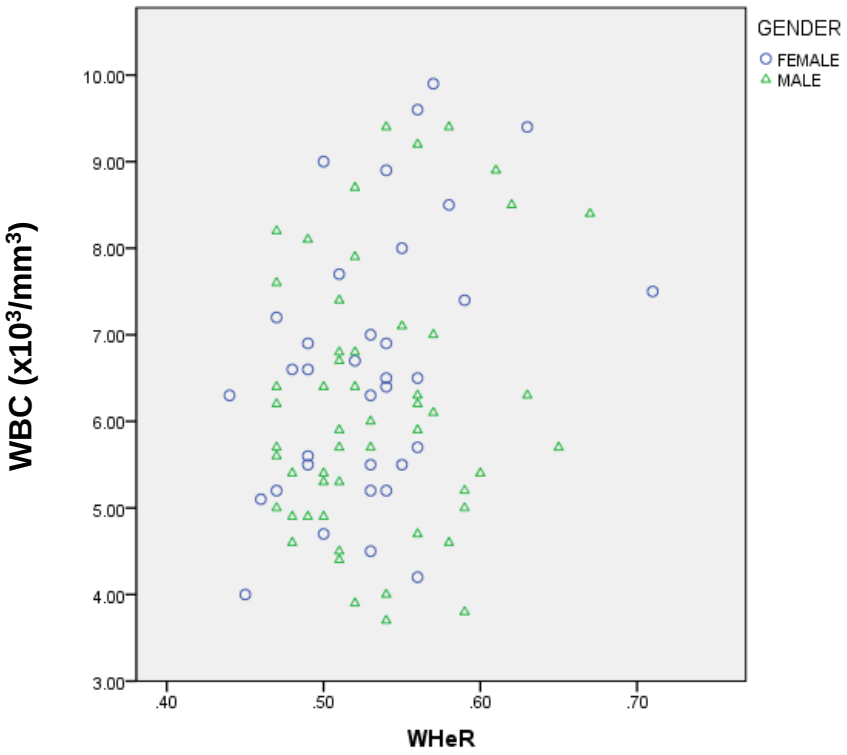

Figure 4.8. Association between white blood cells (WBC) (x10^3/mm^3) and waist-to-height ratio (WHeR) in females (n=34) and males (n = 52). Females: r = 0.414, P = 0.013; males: r = 0.178, P = 0.203.

Table 4.13. Correlation coefficients between indices of obesity and white blood cells.

	BMI	Body fat (%)	WC	WHR	WHeR
WBC	**0.220***	0.203	**0.247***	**0.277****	**0.297****
Neutrophils	**0.248***	**0.267***	**0.255***	**0.235***	**0.309****
Lymphocytes	0.073	**-0.235***	0.115	**0.216***	0.131
Monocytes	**0.235***	0.101	0.193	0.168	**0.260***
Eosinophils	-0.007	0.086	0.099	0.118	0.058
Basophils	0.028	0.105	-0.009	-0.012	-0.002

Partial Pearson correlation adjusted for gender. Significance at *P<0.05, **P<0.01. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; WBC: white blood cells. N = 85.

Table 4.14. Correlation coefficients between indices of obesity and lymphocyte subsets

	BMI	Body Fat (%)	WC	WHR	WHeR
$CD3^+$	0.05	0.011	0.113	**0.276***	0.149
$CD4^+$	0.135	0.119	0.127	0.184	0.139
$CD8^+$	-0.047	-0.089	0.047	**0.259***	0.104
$CD19^+$	-0.086	-0.032	0.006	0.068	-0.033
NK	0.201	0.09	0.05	-0.121	0.042
$CD4^+RA^+$	-0.034	-0.097	-0.033	0.139	0.007
$CD4^+RO^+$	**0.215***	0.182	0.179	0.127	0.169
$CD8^+RA^+$	-0.069	-0.14	0.003	0.23	0.057
$CD8^+RO^+$	0.031	0.077	0.137	**0.216***	0.166
$NKCD3^+$	0.046	-0.014	0.058	0.094	0.047
CD4/CD8	0.089	0.207	0.007	-0.104	-0.021
CD3/CD19	0.168	0.061	0.131	0.181	0.202
CD4RO/CD4RA	0.103	0.08	0.065	-0.055	0.036
CD8RO/CD8RA	-0.157	**-0.243***	-0.120	0.138	-0.048

Partial Pearson correlations adjusted for gender. Significance set at *P<0.05. Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio. N = 85.

4.2.4. Indices of obesity and serum biochemistry, adipokines, and inflammation markers

Moderate significant positive correlations were observed between indices of general and abdominal obesity and glucose, HOMA index, triglycerides, total cholesterol, and VLDL-c (Table 4.15 and Figures 4.9-11). When females and males were tested separately for glucose and waist circumference, only females presented a moderate significant correlation (Figure 4.9). When overweight and obese participants were compared, glucose, triglycerides and VLDL-c levels were significantly higher in the latter (median [Q1, Q3]): glucose: 4.8 [4.5 – 5.1] vs 5.2 [4.8 – 5.3] mmol/L, P = 0.002; triglycerides: 4.3 [3.1 – 6.4] vs 5.4 [4.0 – 8.8] mmol/L, P = 0.037; VLDL-c: 0.9 [0.6 – 1.3] vs 1.1 [0.8 – 1.8] mg/dl, P = 0.041 respectively.

In relation to inflammatory markers, BMI had significant positive associations with leptin, ceruloplasmin, CRP and ICAM1 (Table 4.16 and Figures 4.12-15). When ceruloplasmin and ICAM1 were tested split by gender, only males had significant correlations with BMI (Figures 4.13 & 4.15), and the adhesion molecule presented as well a significant positive correlation with WHR in males (Figure 4.16). When overweight and obese participants were compared, CRP levels were significantly higher in the latter (0.50 [0.17 – 1.53] vs 1.76 [0.55 – 5.88] mg/dl, respectively, P = 0.003).

Table 4.15. Correlation coefficients between indices of obesity and blood biochemistry

	BMI	Body Fat (%)	WC	WHR	WHeR
Glucose	**0.235***	-0.076	**0.345****	**0.238***	**0.243***
Insulin	0.132	0.052	0.133	0.109	0.092
HOMA	**0.282****	0.162	0.138	0.233	**0.259***
NEFA	0.092	0.105	0.05	-0.107	0.032
Triglycerides	**0.303****	0.185	**0.352****	**0.492*****	**0.396*****
Cholesterol	0.175	-0.031	0.19	**0.238****	**0.223***
VLDL-c	**0.302****	0.184	**0.356****	**0.494*****	**0.397*****
LDL-c	0.119	-0.033	0.128	0.133	0.151
HDL-c	-0.054	-0.197	-0.087	-0.087	-0.08
ApoA	-0.006	-0.051	-0.047	-0.016	-0.005
ApoB	0.198	0.159	0.086	0.210*	0.219*

Partial Pearson correlations adjusted for gender. Significance at *P< 0.05, **P<0.01, and ***P<0.001. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; HOMA: homeostatic model assessment; NEFA: non-esterified fatty acids, VLDL-c: very low-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; ApoA: apolipoprotein A; ApoB: apolipoprotein B. N = 85.

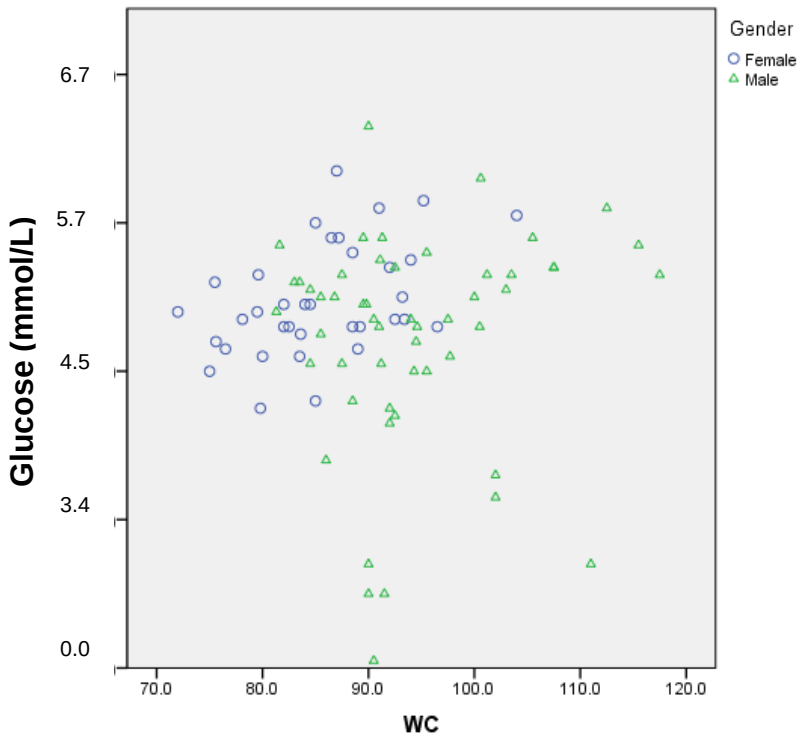

Figure 4.9. Association between glucose (mmol/L) and waist circumference (WC) in females (n=34) and males (n = 52). Females: r = 0.450, P = 0.007; males: r = 0.103, P = 0.463.

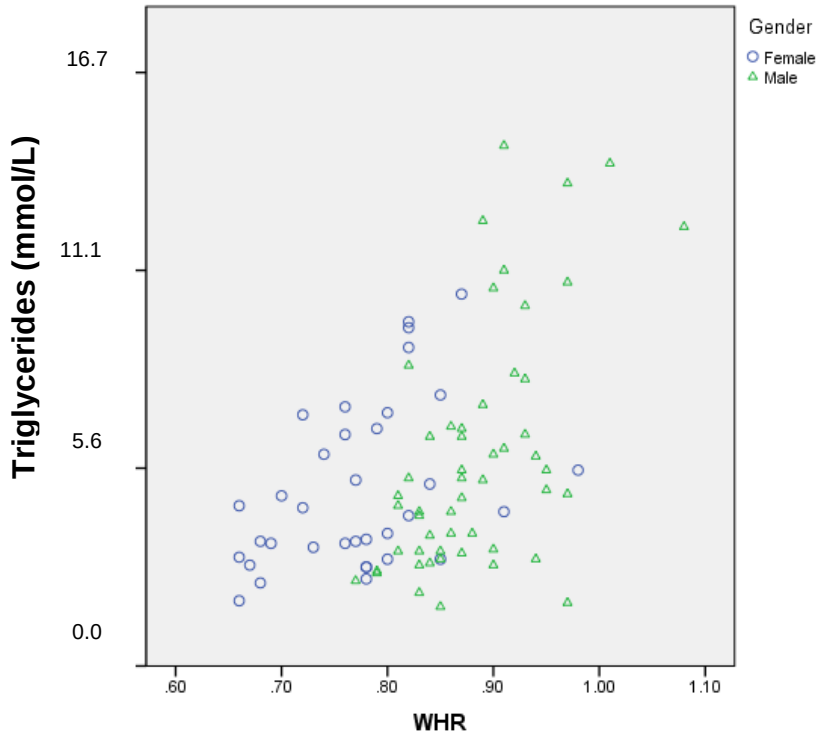

Figure 4.10. Association between triglycerides (mmol/L) and waist-to-hip ratio (WHR) in females (n=34) and males (n = 52). Females: r = 0.419, P = 0.012; males: r = 0.573, P < 0.001.

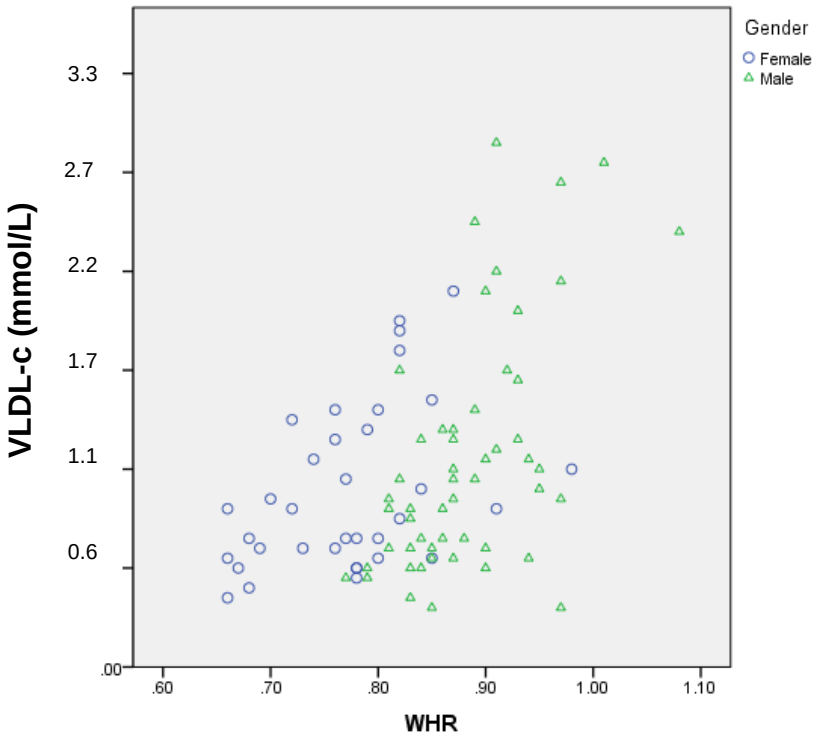

Figure 4.11. Association between VLDL-c (mmol/L) and waist-to-hip ratio (WHR) in females (n=34) and males (n = 52). Females: r = 0.428, P = 0.010; males: r = 0.570, P < 0.001.

Table 4.16. Correlation coefficients between indices of obesity and markers of inflammation

	BMI	Body Fat (%)	WC	WHR	WHeR
Ghrelin	-0.166	-0.317	-0.314	-0.009	-0.091
Leptin	**0.396*** **	0.183	0.184	-0.085	0.101
Adiponectin	-0.156	-0.392	-0.324	-0.03	-0.139
Ceruloplasmin	**0.312****	0.332	0.369	0.394	0.396
CRP	**0.354*** **	0.493	0.098	0.046	0.102
IL-1β	0.166	0.194	-0.295	-0.034	-0.091
IL-2	-0.234	-0.202	-0.28	-0.101	-0.187
IL-6	-0.111	-0.183	-0.133	-0.131	-0.186
IL-8	-0.169	-0.092	-0.236	-0.405	-0.287
IL-10	-0.278	-0.194	-0.257	-0.142	-0.199
GM-CSF	0.193	0.124	0.086	0.335	0.252
IFNγ	0.152	0.075	0.055	0.361	0.242
TNFα	0.253	0.147	0.132	0.418	0.318
VCAM-1	0.357	-0.039	-0.019	0.314	0.342
ICAM-1	**0.232***	0.308	0.375	0.384	0.175

Partial Pearson correlations adjusted for gender. Significance at *P<0.05, **P<0.01, and ***P<0.001. BMI: body mass index; WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio; CRP: C-reactive protein; GM-CSF: granulocyte macrophage colony-stimulating factor; IL: interleukin; IFNγ: interferon gamma; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1; ICAM-1: intracellular adhesion molecule.
IL4 not included in the analysis due to insufficient data. N=91

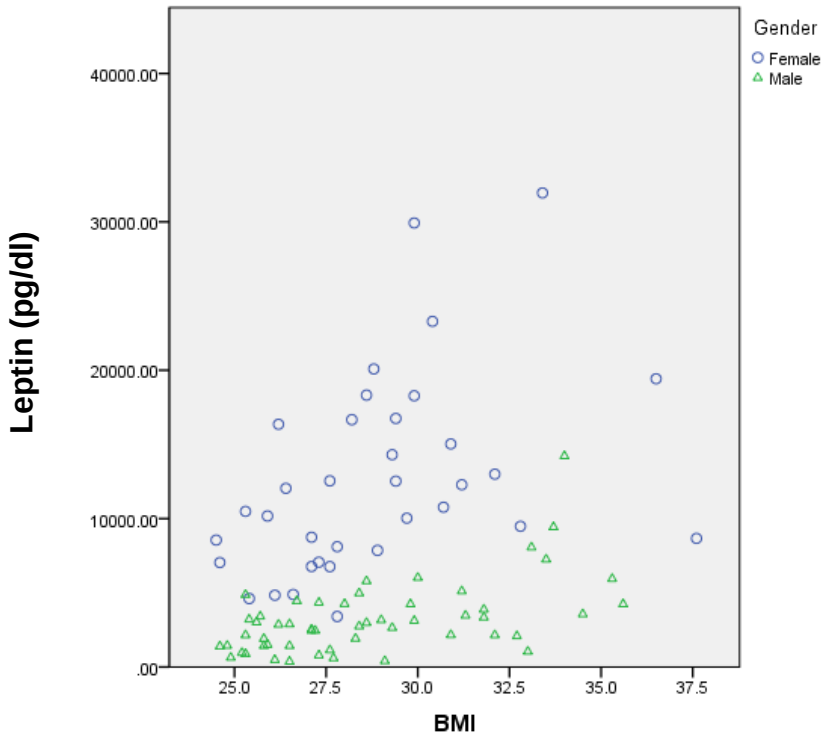

Figure 4.12. Association between leptin (pg/dl) and BMI in females (n=34) and males (n = 52). Females: r = 0.425, P = 0.011; males: r = 0.559, P < 0.001.

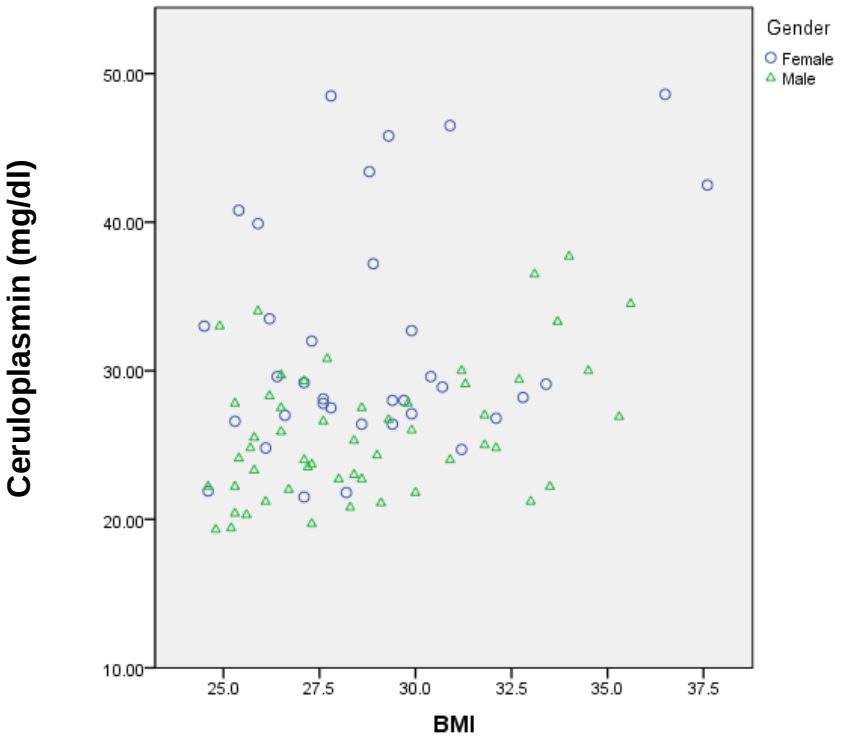

Figure 4.13. Association between ceruloplasmin (mg/dl) and BMI in females (n=34) and males (n = 52). Females: r = 0.293, P = 0.088; males: r = 0.425, P < 0.001.

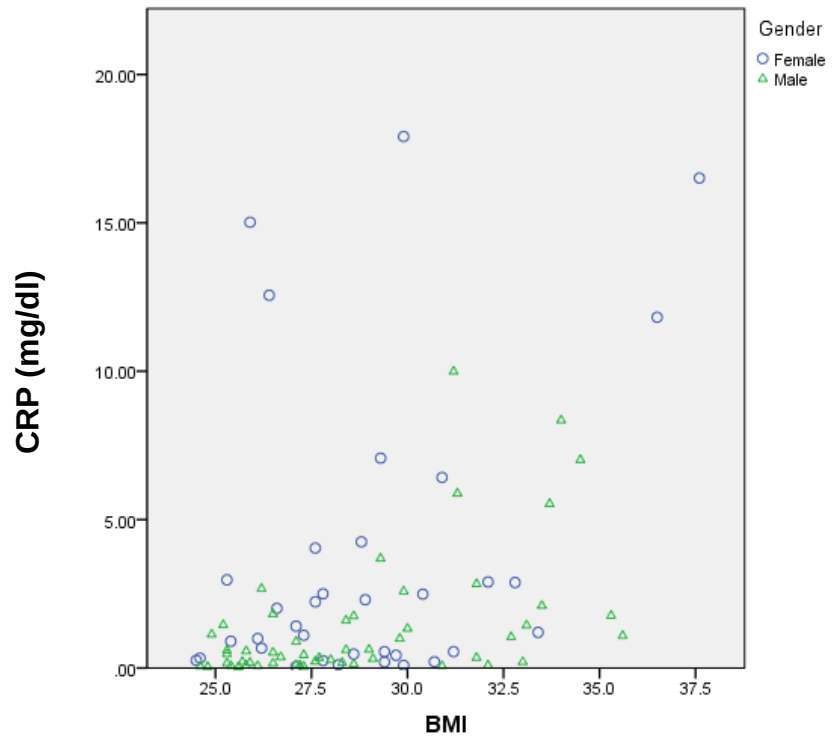

Figure 4.14. Association between C-reactive protein (CRP) (mg/dl) and BMI in females (n=34) and males (n = 52). Females: r = 0.355, P = 0.037; males: r = 0.506, P < 0.001.

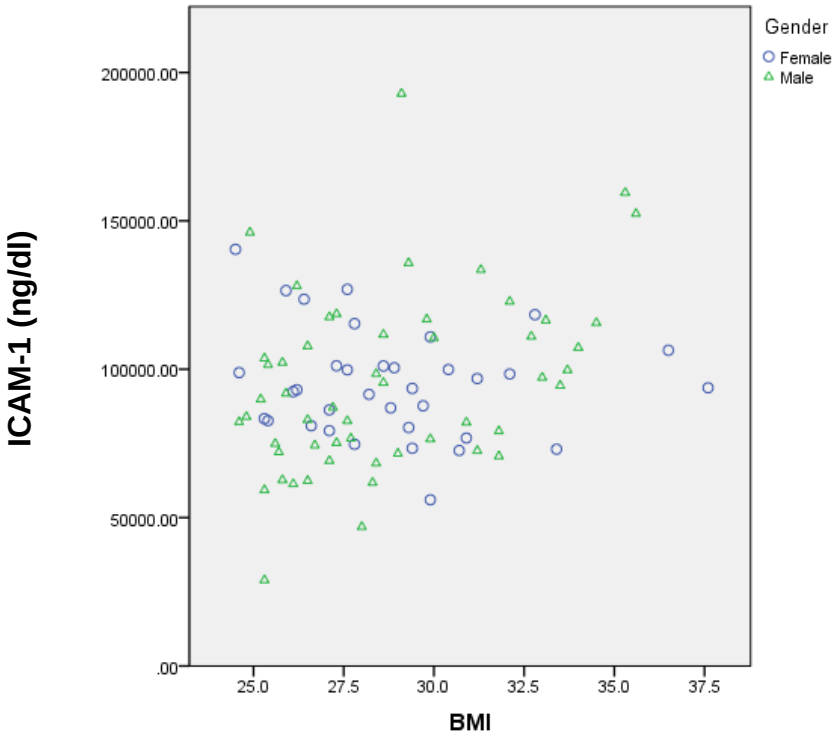

Figure 4.15. Association between ICAM-1 (ng/dl) and BMI in females (n=34) and males (n = 52). Females: r = −0.158, P = 0.363; males: r = 0.390, P = 0.004.

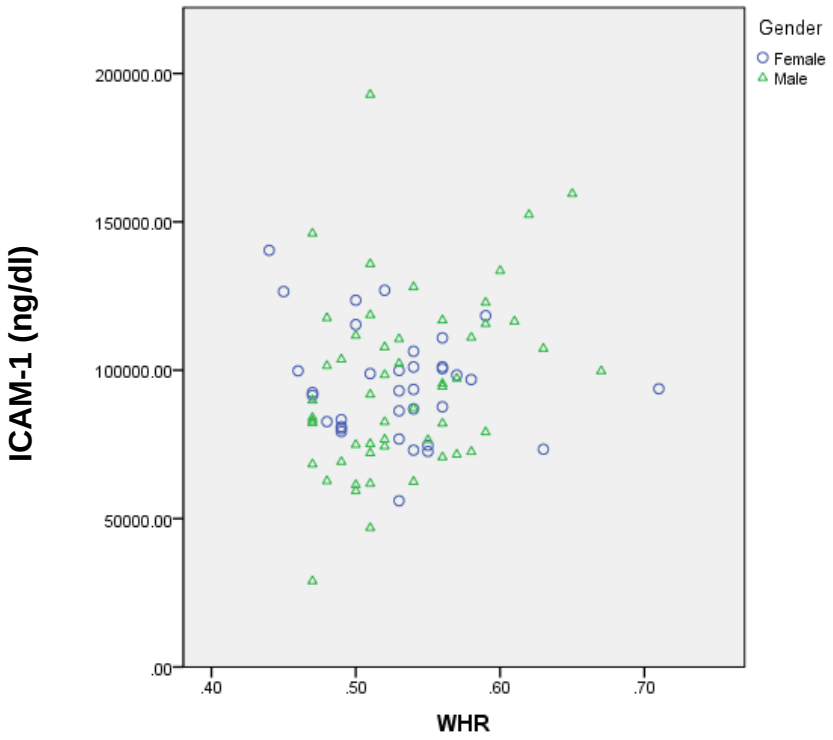

Figure 4.16. Association between ICAM-1 (ng/dl) and waist-to-hip ratio (WHR) in females (n=34) and males (n = 52). Females: r = −0.208, P = 0.231; males: r = 0.340, P = 0.013.

4.2.3 Abdominal obesity and markers of metabolic disease

Different biochemical and hormonal markers were compared between abdominally obese and non-abdominally obese participants. In general, values in the abdominally obese groups were higher than in the non-abdominally obese groups. Triglycerides, VLDL-c, and leptin presented significantly elevated values in the abdominally obese; glucose, total cholesterol, LDL-c, and ApoB presented similar trends. HDL-c, iron, ferritin, and ghrelin presented inconsistent directions of difference between the abdominally obese and the non-abdominally obese, depending on which criterion was used (Table 4.17).

Table 4.17. Average values of biochemical markers in participants classified as abdominally obese (AO) or non-abdominally obese (Non-AO) according to indicators.

	WAIST CIRCUMFERENCE			WAIST-TO-HIP RATIO			WAIST-TO-HEIGHT RATIO		
	Non-AO (N=39)	**AO (N=49)**	***P***	**Non-AO (N=81)**	**AO (N=7)**	***P***	**Non-AO (N=22)**	**AO (N=66)**	***P***
Glucose (mmol/L)*	4.5±0.1	4.9±0.1	0.087	4.7±0.1	5.2±0.1	0.259	4.6±0.1	4.8±0.1	0.197
Insulin (pmol/l)*[1]	12.7±2.8	12.7±2.0	0.956	12.5±2.3	14.2±1.6	0.767	11.0±2.2	13.2±2.3	0.493
HOMA index*[1]	0.4±2.9	0.5±2.2	0.696	0.4±2.5	0.5±1.5	0.733	0.4±2.2	0.5±2.5	0.375
NEFA (mmol/L)[†]	0.02±0.01	0.02±0.01	0.872	0.02±0.01	0.02±0.01	0.682	0.02±0.07	0.02±0.07	0.682
Triglycerides (mmol/L)*	4.2±0.1	5.3±0.1	**0.009**	4.65±0.1	7.2±0.1	**0.016**	**3.8±0.1**	**5.2±0.1**	**0.009**
Total cholesterol (mmol/L)	8.8±1.7	9.6±1.8	0.203	9.1±1.8	10.2±1.3	0.291	8.8±1.8	9.4±1.8	0.127
VLDL-c (mmol/L)*	**0.8±0.1**	**1.1±0.1**	**0.010**	**0.9±0.1**	**1.4±0.1**	**0.014**	**0.8±0.1**	**1.0±0.1**	**0.011**
LDL-c (mmol/L)[#]	5.3±1.3	5.6±1.35	0.473	5.3±0.1	5.7±0.1	0.644	5.3±1.4	5.7±1.3	0.274
HDL-c (mmol/L)*	2.4±0.1	2.6±0.1	0.407	2.5±0.1	2.7±0.1	0.835	2.6±0.1	2.5±0.1	0.677
ApoA (mmol/L)*	7.9±0.1	7.9±0.1	0.451	7.9±0.1	8.5±0.1	0.511	8.1±0.1	7.9±0.1	0.548
ApoB (mmol/L)*	4.4±0.1	4.7±0.2	0.245	4.4±0.1	4.9±0.1	0.244	4.2±0.1	4.6±0.1	0.128
Iron (mg/dl)*	86.6±1.5	79.8±1.7	0.951	85.0±1.5	60.6±1.7	0.123	81.7±1.6	83.2±1.5	0.987
Ferritin (ng/ml)*	63.86±2.6	53.03±3.6	0.128	60.1±3.1	35.4±3.4	0.984	42.8±2.7	63.6±3.3	0.190
Transferrin (mg/dl)[#]	237.6±44.4	259.4±51.0	0.460	242.6±1.3	262.4±1.19	0.926	248.2±50.4	250.3±51.5	0.600
Adiponectin (ng/ml)*	4365.2±0.0	4786.3±0.0	0.842	4786.3±0.0	3388.4±0.0	0.081	4073.8±0.0	4786.3±0.0	0.227
Ghrelin (pg/ml)[2]*	288.40±1.5	263.03±1.5	0.418	269.15±1.5	331.13±1.6	0.228	269.15±1.4	275.42±1.6	0.728
Leptin (ng/ml)[3]*	**2.57±0.0**	**6.65±0.0**	**<0.001**	4.07±0.0	8.51±0.0	0.934	**3.31±0.0**	**4.68±0.0**	**0.005**

HOMA: homeostatic model assessment; NEFA: non-esterified fatty acids, VLDL-c: very low-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; ApoA: apolipoprotein A; ApoB: apolipoprotein B. [1]Valid n: For waist circumference: 19 (Non-AO) and 38 (AO); for WHR: 52 (Non-AO) and 5 (AO); for WHeR: 12 (Non-AO) and 45 (AO). [2]Valid n: For Waist circumference: 38 (Non-AO) and 49 (AO); for WHR: 80 (Non-AO) and 7 (AO); for WHeR: 22 (Non-AO) and 65 (AO). [3]Valid n: for waist circumference: 39 (Non-AO) and 47 (AO); for WHR 80 (Non-AO) and 6 (AO); for WHeR: 22 (Non-AO); and 64 (AO). *Values are presented as geometric means ±SD for all criteria. #Values are presented as geometric means ±SD for comparisons based on WHR; [†]Values are presented as geometric means ±SD for comparisons based on WHeR.

4.3. Effect of the treatments

4.3.1. On the indices of obesity

Female participants in group 1 presented significant reductions in waist circumference with both treatment B1 and the placebo. Females in group 2 lost weight with both the treatment B2 and the placebo, but a reduction in BMI was significant only with the treatment, and reductions in WHR and WHeR were significant only with placebo (Table 4.18).

Male participants in group 1 presented significant reductions in weight and BMI with the treatment B1, and in waist circumference, WHR, and WHeR with both treatment and placebo. In group 2, male participants presented significant reductions in abdominal obesity markers only under the treatment B2 (Table 4.19).

When the interactions between time-point and type of drink and were analysed, no significant differences were observed between baseline and post-treatment, or between placebo and functional drinks, for any of the variables studied, in females and males separately as well as in the whole sample (Supplementary annex 9 & 10).

4.3.2. On the red blood cell series

No evident effects were observed in the participants after the consumption of treatments B1 and B2 (Tables 4.20 and 4.21), except for relative changes in haemoglobin concentration in red cells (MCH) in males, where all drinks but treatment B1 were associated with an increase (Figure 4.17).

The analysis of the interactions between time-point and type of drink confirmed the lack of significant differences between baseline and post-treatment, and between placebo and functional drinks, for most of the variables studied. In both females and males, transferrin levels were significantly different between baseline and post-treatment; in males only, iron levels were different between placebo and functional drinks. These differences were not observed in the whole sample except transferrin (Supplementary annex 9 & 10).

4.3.3. On the white blood cells

In female participants, treatment B2 had a significant effect on white blood cells, in particular, it reduced neutrophil counts (Tables 4.22) No changes were observed in lymphocyte subsets (Table

4.24). In male participants, treatment B2presented significant reductions in total white blood cell counts and neutrophil counts (Table 4.23; Figures 4.18 & 4.19), total T helper cells ($CD4^+$) and naive T helper cells ($CD4^+RA^+$) (Table 4.25; Figures 4.20 & 4.21).

In males, the analysis of the interactions between time-point and type of drink confirmed significant differences in white blood cells ($P = 0.047$, and detected differences in lymphocytes, eosinophils and basophils ($P < 0.001$), between placebo and functional drinks, but not between baseline and post-treatment values. In females, $CD4^+RA^+$ and $CD4^+RO^+$ were significantly different between placebo and functional drinks, but not between baseline and post-treatment. When the whole sample was tested, the significant difference remained only for basophils (Supplementary annex 9 & 10).

4.3.4. On the serum and plasma variables

In females, both treatment B2 and placebo caused significant reductions in glucose and HDL-c, but only B2 reduced as well total cholesterol, LDL-c, and ApoA levels (Table 4.26). In males, both B2 and placebo caused significant increases in NEFA; B2 also caused a significant increase in glucose, while placebo saw a significant increase in VLDL-c and a decrease in ApoB (Table 4.27). The analysis of the interactions between time-point and type of drink confirmed significant differences in glucose and total cholesterol between placebo and functional drinks in females, and in NEFA in males. In the whole sample, NEFA were significantly higher at the end of the treatment, but no differences were detected between placebo and functional drinks (Supplementary annex 9 & 10).

In relation to markers of inflammation, in females ghrelin increased after all treatments, and leptin increased after all drinks but B1; similarly, only B1 reduced IL-6, while VCAM-1 and IL-8 increased under the placebo (Table 4.28). In males, leptin levels increased significantly with placebos but not with treatments; VCAM-1 decreased with all treatments but B2; finally, IL-8 presented a significant the reduction with treatment B1 (Table 4.29).

When males and females were tested separately males presented significant difference between baseline and post treatment levels in NEFA and Transferrin ($P < 0.05$), Iron between placebo and functional drink ($P < 0.05$) and for VCAM1 and ICAM1 for the interaction between drink type and baseline/ post treatment ($P < 0.05$ for both) (Annex 10). For females, Ghrelin, Leptin, Glucose, Total cholesterol, and Transferrin presented significant different vales between baseline and post treatment ($P \leq 0.001$, $P < 0.005$, $P < 0.005$, $P < 0.05$, $P < 0.05$, respectively) (Annex 10). IL-1β and TNA- α in

females and VCAM1 and ICAM1 presented significance for the interaction of placebo/functional and baseline/post treatment, but not separately ($P < 0.05$ for all).

In the whole sample, leptin, VCAM1 and ICAM1 levels were significantly different between baseline and post–treatment, irrespective of the type of drink consumed (Supplementary annex 9 & 10).

Table 4.18. Effect of the treatments on indices of obesity in female participants.

	Group 1				Group 2			
	B1 (N=17)		P1 (N=19)		B2 (N=18)		P2 (N=16)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Weight (kg)	77.5 ± 8.1	77.1 ± 8.2	77.9 ± 8.1	77.3 ± 7.9	**73.0 ± 8.3**	**72.3 ± 8.4***	**71.3 ± 7.3**	**70.8 ± 7.3***
BMI	28.8 (27.7 – 29.9)	28.6 (27.0 – 30.9)	28.9 (27.0 – 31.4)	28.90 (27.1 – 31.3)	**27.6 (26.3 – 29.1)**	**27.3 (25.7 – 29.0)***	27.15 (25.8 – 28.6)	27.0 (25.5 – 28.7)
Body Fat (%)	38.0 ± 3.3	37.8 ± 4.0	37.9 ± 4.0	36.9 ± 4.3	36.5 ± 4.5	35.5 ± 4.8	36.2 ± 4.1	35.5 ± 3.9
Waist (cm)	**85.0 (80.0 – 91.2)**	**84.2 (77.4 – 89.5)***	**85.5 (79.5 – 89.2)**	**83.0 (79.6 – 90.5)***	84.5 (80.2 – 88.5)	83.2 (80.4 – 88.0)	83.8 (79.7 – 88.1)	84.3 (79.0 – 86.0)
WHR	0.76 ± 0.07	0.75 ± 0.07	**0.76 ± 0.07**	**0.75 ± 0.07***	0.78 ± 0.08	0.77 ± 0.07	**0.78 ± 0.07**	**0.77 ± 0.07***
WHeR	**0.53 (0.49 – 0.56)**	**0.51 (0.48 – 0.54)***	0.51 (0.49 – 0.56)	0.49 (0.49 – 0.56)	0.53 (0.49 – 0.55)	0.53 (0.49 – 0.54)	**0.53 (0.49 – 0.55)**	**0.52 (0.48 – 0.54)***

Table 4.19. Effect of the treatments on indices of obesity in male participants.

	Group 1				Group 2			
	B1 (N=23)		P1 (N=26)		B2 (N=26)		P2 (N=25)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Weight (kg)	**89.5 ± 10.7**	**88.7 ± 11.1***	89.5 ± 10.6	89.0 ± 11.4	91.3 ± 13.1	90.3 ± 13.4	90.7 ± 13.3	90.7 ± 13.5
BMI	**27.7 (25.9 – 31.2)**	**27.3 (25.8 – 31.2)***	27.3 (25.8 – 31.3)	27.2 (25.7 – 30.5)	28.3 (26.2 – 31.1)	27.7 (26.0 – 31.1)	27.6 (25.8 – 31.8)	27.5 (26.2 – 31.4)
Body Fat (%)	23.3 ± 5.0	22.7 ± 6.1	23.9 ± 4.7	23.4 ± 5.3	23.7 ± 5.2	23.3 ± 5.8	23.5 ± 5.1	23.0 ± 5.9
Waist (cm)	**92.4 (87.5 – 99.8)**	**90.4 (86.8 – 98.1)***	**91.3 (88.5 – 100.0)**	**90.5 (87.2 – 99.2)***	**93.3 (89.8 – 101.5)**	**91.5 (86.7 – 99.8)****	91.0 (86.8 – 100.6)	90.7 (85.8 – 100.0)
WHR	**0.88 ± 0.06**	**0.87 ± 0.06***	**0.88 ± 0.06**	**0.87 ± 0.06***	**0.89 ± 0.07**	**0.88 ± 0.07***	0.88 ± 0.07	0.88 ± 0.07
WHeR	**0.52 (0.49 – 0.57)**	**0.51 (0.48 – 0.57)***	**0.52 (0.49 – 0.57)**	**0.52 (0.48 – 0.56)***	**0.52 (0.50 – 0.58)**	**0.52 (0.48 – 0.56)****	0.52 (0.49 – 0.56)	0.52 (0.48 -0.56)

Data presented as the mean ± SD or median (Q1, Q3). Bold characters indicate significant differences between baseline and endpoint values at *P < 0.05, **P < 0.01. Weight, body fat, and WHR analysed by the paired-*t* test. BMI, waist and WHeR analysed by Wilcoxon. BMI: body mass index; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio.

Table 4.20. Effect of the treatments on red blood series in female participants.

	Group 1				Group 2			
	B1 (N=19)		P1 (N=19)		B2 (N=19)		P2 (N=17)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
RBC ($x10^6$cells/mm^3)	4.44±0.30	4.48±0.43	4.44±0.41	4.41±0.43	4.43±0.40	4.35±0.41	4.48±0.33	4.44±0.29
Hb (g/dl)	13.3 (13.0 – 13.5)	13.3 (12.6 – 14.0)	13.0 (12.4 – 13.7)	12.9 (12.4 – 13.7)	12.8 (11.9 – 13.4)	12.9 (11.2 – 13.3)	13.2 (12.5 – 13.9)	12.9 (12.7 – 13.9)
Haematocrit (%)	40.2±2.2	40.4±3.3	39.6±3.1	40.0±3.1	39.5±3.1	38.8±3.5	40.1±3.3	40.1±3.4
MCV (fl)	92.0 (87.0 – 94.0)	91.5 (88.0 – 93.0)	**90.0 (85.0 – 93.0)**	**92.0 (87.0 – 94.0)***	90.0 (88.0 – 92.0)	91.0 (86.0 – 92.0)	89.0 (88.0 – 92.0)	91.0 (86.0 – 93.0)
MCH (pg)	30.3 (28.7 – 30.7)	30.1 (28.5 – 31.0)	29.4 (28.3 – 30.2)	30.1 (27.8 – 31.6)	28.9 (27.6 – 29.9)	29.3 (26.6 – 31.1)	29.5 (28.1 – 30.0)	29.7 (28.3 -30.4)
MCHC (g/dl)	**32.8±1.1**	**33.0±1.0****	32.9±1.1	32.8±1.0	32.4±0.7	32.6±1.2	32.7±0.9	32.8±1.1
Anisocytosis Index (%)	12.6 (12.0 – 13.7)	12.7 (12.2 – 13.4)	12.8 (12.0 – 13.7)	12.9 (12.6 – 13.6)	12.5 (11.8 – 13.5)	12.5 (12.0 – 13.1)	12.2 (11.8 – 12.9)	13.0 (11.9 – 13.6)
Platelets ($x10^3$/mm^3)	272.7±51.7	276.6±42.5	273.7±53.4	278.5±59.4	264.5±53.5	266.4±68.77	**266.1±34.4**	**253.1±48.0***
MPV (fl)	9.1 (8.6 – 9.7)	8.9 (8.7 – 9.5)	8.7 (8.3 – 9.9)	9.0 (8.4 – 9.6)	9.10 (8.3 – 9.5)	8.9 (8.4 – 9.3)	9.2 (8.6 – 9.6)	9.1 (8.4 – 9.3)
Iron (mg/dl)	71.0 (53.0 – 98.0)	65.5 (39.0 – 103.0)	63.5 (43.0 – 80.0)	47.0 (40.0 – 74.0)	80.26±38.37	71.11±42.49	79.06±36.21	82.35±41.73
Ferritin (ng/dl)	36.0 (16.0 – 51.0)	37.5 (22.5 – 60.5)	27.5 (13.0 – 46.0)	28.0 (20.0 – 37.0)	23.0 (13.0 – 40.0)	17.0 (10.0 – 34.0)	25.0 (14.0 – 49.0)	27.0 (14.0 – 40.0)
Transferrin (mg/dl)	255.2±63.2	255.3±48.2	258.0±50.7	247.1±56.2	**266.5±42.6**	**233.9±53.0****	**257.0 (240.0 – 288.0)**	**224.0** (208.0 – 268.0)**

Data presented as the mean ± SD or median (Q1, Q3). Baseline and final values compared by the paired-*t* test or Wilcoxon test, depending on normality. Significance at *P < 0.05, **P < 0.01. RBC: red blood cells; Hb: haemoglobin; MCHC: mean corpuscular Hb concentration; MCV: mean corpuscular volume; MCH: mean corpuscular Hb, MPV: mean platelet volume.

Table 4.21. Effect of the treatments on red blood series in male participants.

	Group 1				Group 2			
	B1 (N=23)		P1 (N=26)		B2 (N=26)		P2 (N=25)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
RBC ($x10^6$cells/mm^3)	5.06±0.48	5.03±0.39	5.09±0.98	5.00±0.51	5.03±0.30	5.02±0.31	5.08±0.30	5.04±0.26
Hb (g/dl)	15.0 (14.2 – 15.5)	15.2 (13.8 – 15.7)	14.8 (14.4 – 15.6)	15.1 (14.2 – 15.7)	**14.8 (14.3 – 15.2)**	**15.0 (14.7 – 15.7)***	15.0 (14.3 – 15.7)	15.1 (14.5 – 15.8)
Haematocrit (%)	44.8±3.0	44.9±3.1	45.4±3.3	44.8±3.4	44.7±2.9	45.1±5.5	45.5±3.0	45.4±3.0
MCV (fl)	90.0 (86.0 – 93.0)	90.0 (88.0 – 95.0)	91.0 (88.0 – 93.0)	90.0 (89.0 – 93.0)	90.0 (86.0 – 92.0)	89.5 (87.0 – 92.0)	**91.0 (89.0 – 92.0)**	**90.0 (88.0 – 93.0)***
MCH (pg)	30.1 (28.9 – 31.0)	29.9 (29.2 – 30.8)	**29.9 (28.9 – 31.1)**	**30.7 (29.3 – 31.9)***	**29.4 (28.6 – 30.2)**	**29.8 (29.4 – 31.1)****	30.0 (28.8 – 30.5)	30.0 (28.9 – 30.7)
MCHC (g/dl)	33.3±1.0	33.0±1.0	33.0±0.8	33.6±1.0	33.1±1.1	33.5±0.9	33.0±1.1	33.0±0.7
Anisocytosis Index (%)	12.6±1.2	12.9±1.1	12.7 (12.1 – 13.3)	13.3 (12.0 – 13.7)	12.7 (11.9 – 13.2)	13.0 (12.1 – 13.8)	12.3 (12.1 – 13.0)	12.6 (12.1 – 13.2)
Platelets ($x10^3$/mm^3)	237.0 (210.0 – 271.0)	232.0 (205.0 – 276.0)	245.0±44.3	244.6±57.2	232.27±54.7	232.75±58.0	234.5±55.9	231.2±68.3
MPV (fl)	9.0 (8.6 – 9.9)	9.1 (8.6 – 9.9)	9.1 (8.4 – 9.6)	9.2 (8.8 – 9.6)	9.2 (8.4 – 9.7)	9.2 (8.6 – 10.1)	9.2 (8.6 – 9.9)	9.0 (8.7 – 9.9)
Iron (mg/dl)	85.0 (67.0 – 102.0)	83.0 (69.0 – 123.0)	105.0 (92.0 – 119.0)	96.0 (75.0 – 100.0)	94.27±33.64	85.8±21.7	93.2±32.5	100.3±35.7
Ferritin (ng/dl)	133.0 (86.0 – 200.0)	140.0 (90.0 – 203.0)	142.5 (102.0 – 175.0)	135.0 (72.0 – 188.0)	119.5 (52.0 – 194.0)	106.5 (59.5 – 170.5)	96.0 (57.0 – 170.0)	117.0 (72.0 – 166.0)
Transferrin (mg/dl)	239.4±38.6	251.7±29.0	**241.0±25.9**	**254.0±26.3***	245.5±43.7	261.5±35.9	248.0 (235.0 – 268.0)	254.0 (236.0 – 278.0)

Data presented as the mean ± SD or median (Q1, Q3). Baseline and final values compared by the paired-*t* test or the Wilcoxon test, depending on normality. Significance at *P < 0.05, **P < 0.01. RBC: red blood cells; Hb: haemoglobin; MCHC: mean corpuscular Hb concentration; MCV: mean corpuscular volume; MCH: mean corpuscular Hb, MPV: mean platelet volume.

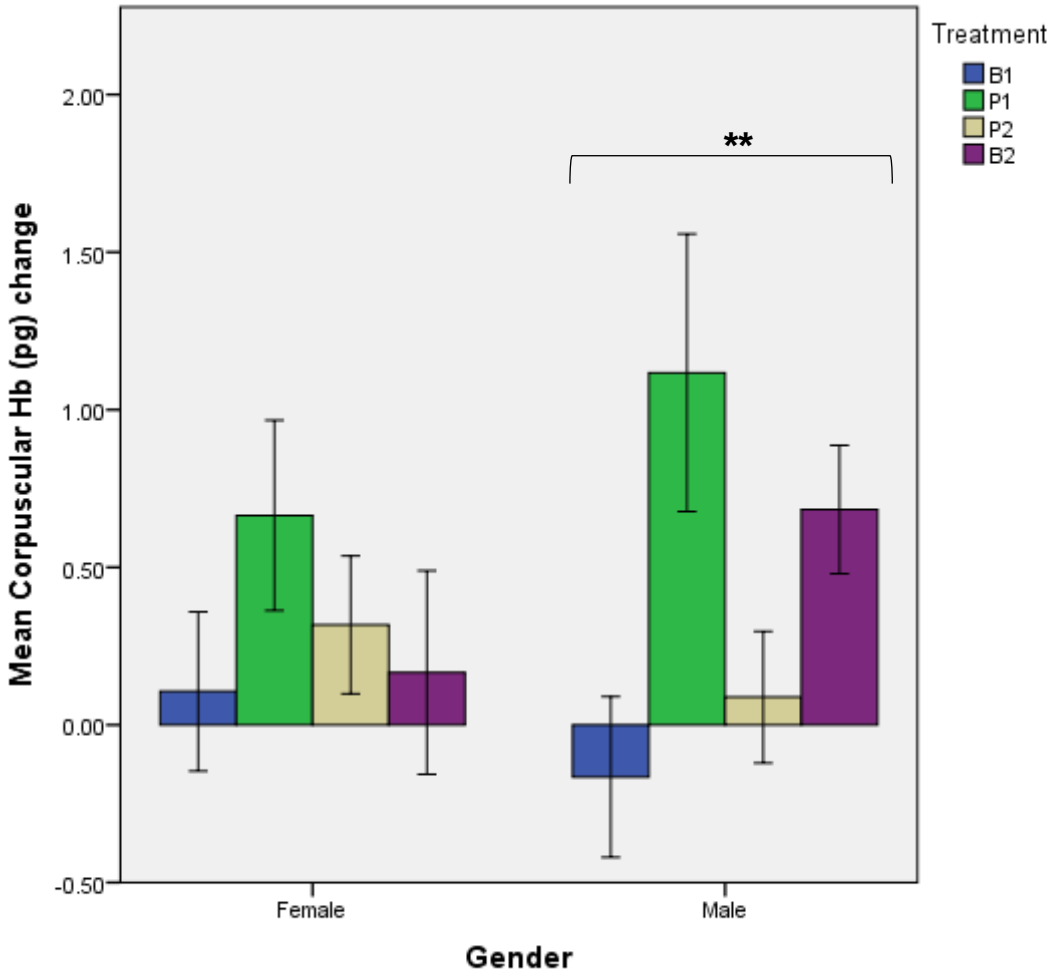

Figure 4.17. Mean corpuscular Hb (pg) change observed in participants according to treatment. Females: n = 19 for B1, P1, and B2; n = 17 for B2; males: n = 23 for B1, n = 26 for P1, n = 25 for P2, n = 26 for B2. Both phases of treatment have been combined. ** Significant difference between treatments at $P < 0.05$, as analysed by the Kruskal-Wallis test. Error bars: +/- 1 SD.

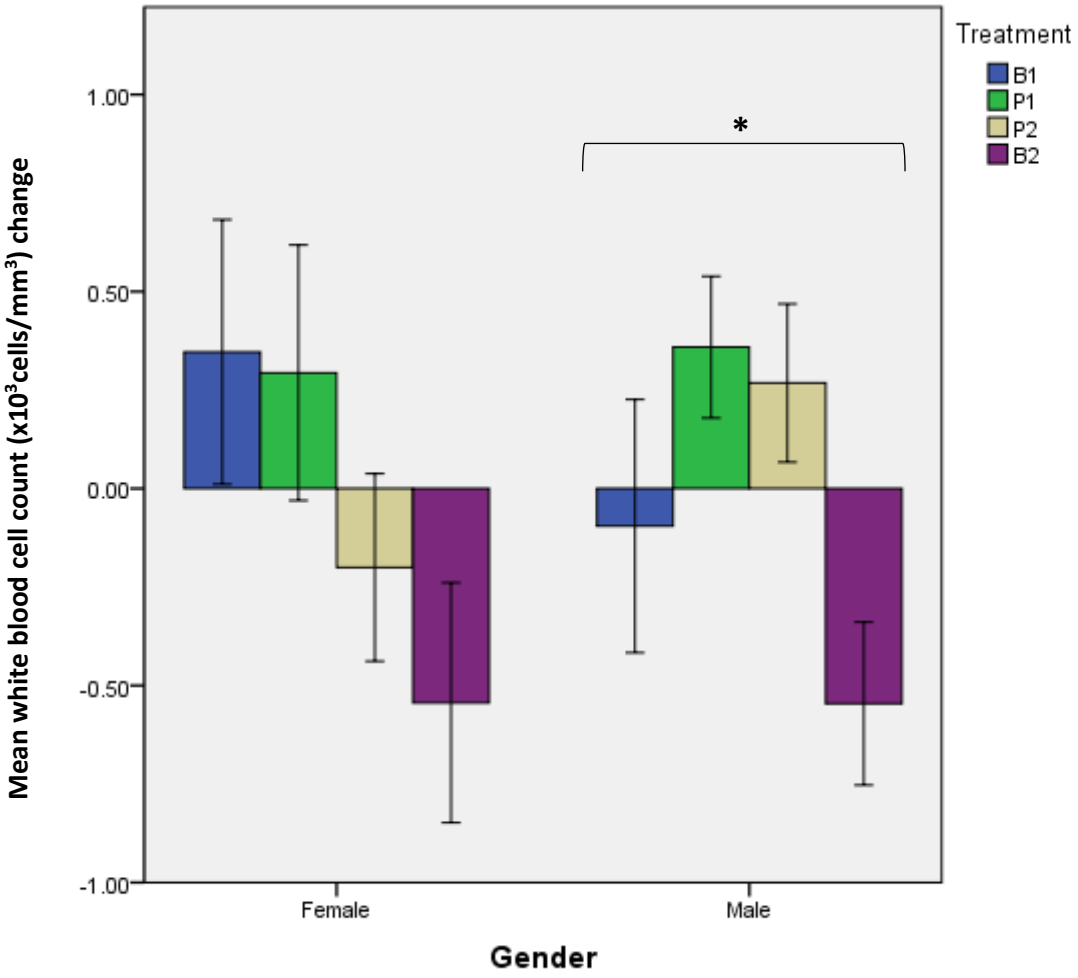

Figure 4.18. White blood cell count (x10^3cells/mm^3) change observed in participants according to treatment. Females: n = 17 for B1, P1, and B2; n = 15 for P2; males: n = 22 for B1, n = 25 for P1 and P2, n = 26 for B2. Both phases of treatment have been combined. * Significant difference between drinks at $P < 0.05$, as analysed by the ANOVA test. Error bars: +/- 1 SD.

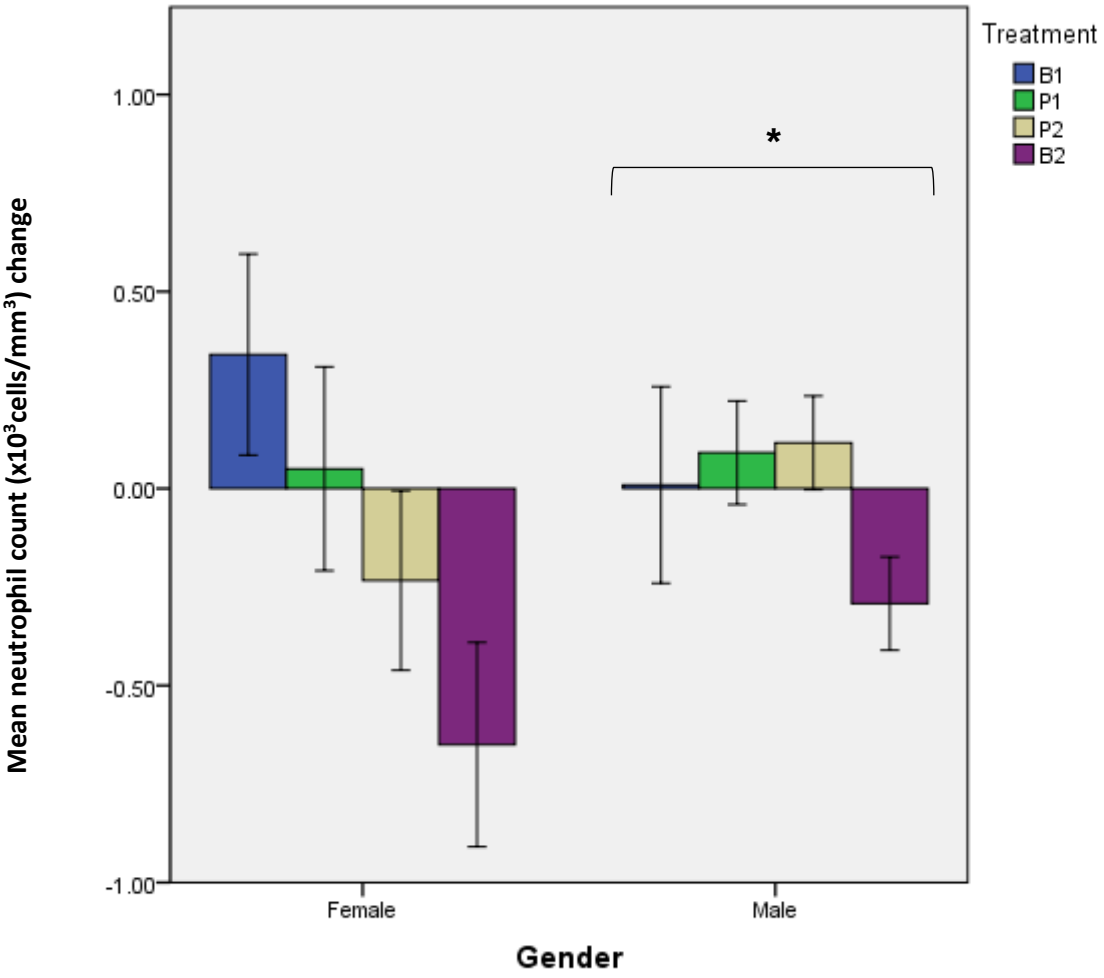

Figure 4.19. Neutrophil count (x10^3cells/mm^3) change observed in participants according to treatment consumed. Females: n = 17 for B1, P1, and B2; n = 15 for P2; males: n = 22 for B1, n = 25 for P1 and P2, n = 26 for B2. Both phases have been combined. * Significant between treatments at $P < 0.05$, as analysed by the ANOVA test. Error bars: +/- 1 SD.

Table 4.22. Effect of the treatments on white blood cells in female participants.

	Group 1				Group 2			
Cell count ($x10^3/mm^3$)	**B1 (N=17)**		**P1 (N=17)**		**B2 (N=17)**		**P2 (N=15)**	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
White Blood Cells	5.85 (4.85 – 7.35)	6.10 (5.10 – 7.10)	6.50 (5.35 – 7.05)	6.70 (5.95 – 7.40)	7.20 (6.30 – 8.30)	6.35 (5.70 – 7.65)	6.70 (5.80 – 7.70)	6.10 (5.70 – 7.20)
Neutrophils	2.85 (2.60 – 3.95)	3.20 (2.60 – 5.00)	3.45 (2.90 – 3.75)	3.50 (3.05 – 3.95)	**4.40 (3.40 – 5.00)**	**3.20 (2.90 – 4.55)***	3.70 (2.80 – 4.70)	3.10 (2.90 – 3.70)
Lymphocytes	2.25 (1.80 – 2.60)	2.20 (1.60 – 2.60)	2.30 (1.90 – 2.60)	2.35 (1.85 – 3.00)	2.20 (2.00 – 2.60)	2.40 (2.10 – 2.60)	2.30 (2.00 – 2.50)	2.20 (1.80 – 2.70)
Monocytes	0.45 (0.30 – 0.55)	0.50 (0.40 – 0.50)	0.50 (0.40 – 0.50)	0.50 (0.35 – 0.55)	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.55)	0.40 (0.40 – 0.60)	0.40 (0.40 – 0.50)
Eosinophils	0.10 (0.10 – 0.25)	0.20 (0.10 – 0.30)	0.15 (0.10 – 0.20)	0.20 (0.10 – 0.25)	0.50 (0.10 – 0.30)	0.20 (0.10 – 0.25)	0.20 (0.10 – 0.30)	0.10 (0.10 – 0.30)
Basophils	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.10)

Data presented as median (Q1, Q3). Baseline and endpoint values compared by the Wilcoxon test. Significance at *P < 0.05.

Table 4.23. Effect of the treatments on white blood cells in male participants.

	Group 1				Group 2			
Cell count ($x10^3/mm^3$)	**B1 (N=22)**		**P1 (N=25)**		**P2 (N=25)**		**B2 (N=26)**	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
White Blood Cells	6.25 (5.90 – 7.60)	6.30 (4.90 – 7.70)	6.20 (5.30 – 7.20)	6.20 (5.50 – 7.10)	5.40 (4.90 – 6.50)	6.00 (4.90 – 6.70)	**5.95 (5.40 – 7.10)**	**5.65 (4.90 – 6.20)***
Neutrophils	3.35 (2.80 – 4.00)	3.40 (2.50 – 3.80)	3.10 (2.60 – 3.90)	3.25 (2.50 – 3.60)	3.10 (2.40 – 3.50)	3.30 (2.50 – 3.90)	**3.15 (2.90 – 4.00)**	**2.85 (2.50 – 3.25)***
Lymphocytes	2.25 (1.90 – 2.70)	2.25 (1.80 – 2.80)	**2.20 (1.70 – 2.60)**	**2.30 (2.00 – 3.00)***	1.90 (1.70 – 2.20)	2.00 (1.80 – 2.40)	2.20 (2.00 – 2.60)	2.10 (1.80 – 2.35)
Monocytes	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.40 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.50 (0.40 – 0.60)	0.40 (0.40 – 0.50)
Eosinophils	0.20 (0.10 – 0.30)	0.20 (0.10 – 0.30)	0.20 (0.10 – 0.20)	0.20 (0.10 – 0.20)	0.10 (0.10 – 0.20)	0.20 (0.10 – 0.20)	0.20 (0.10 – 0.20)	0.15 (0.10 – 0.20)
Basophils	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)	0.00 (0.00 – 0.00)

Data presented as median (Q1, Q3). Baseline and endpoint values compared by the Wilcoxon test. Significance at *P < 0.05.

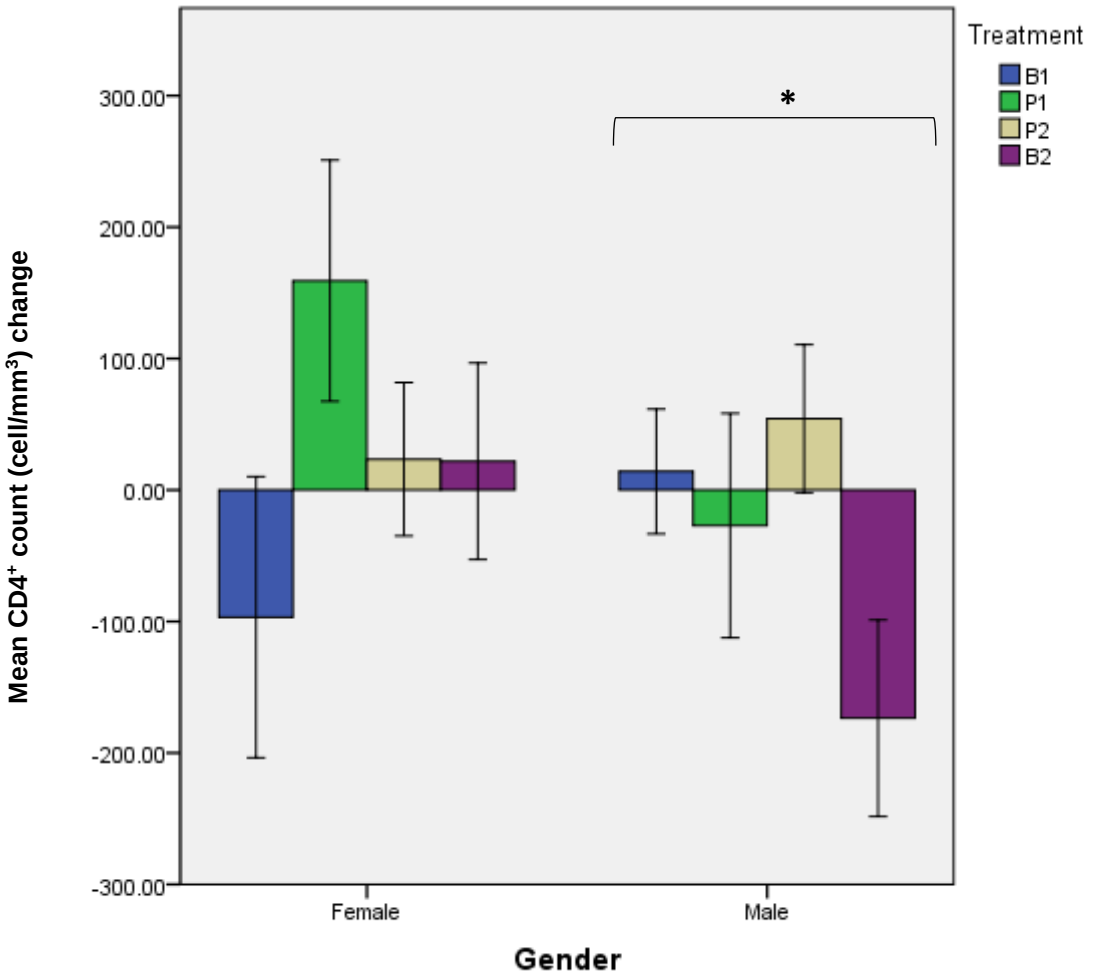

Figure 4.20. $CD4^+$ count (cells/mm^3) change observed in participants according to treatment consumed. Females: n = 17 for B1, P1, and B2; n = 15 for P2; males: n = 22 for B1, n = 25 for P1 and P2, n = 26 for B2. Both phases have been combined. * Significant at $P < 0.05$, as analysed by the Kruskal-Wallis test. Error bars: +/- 1 SD.

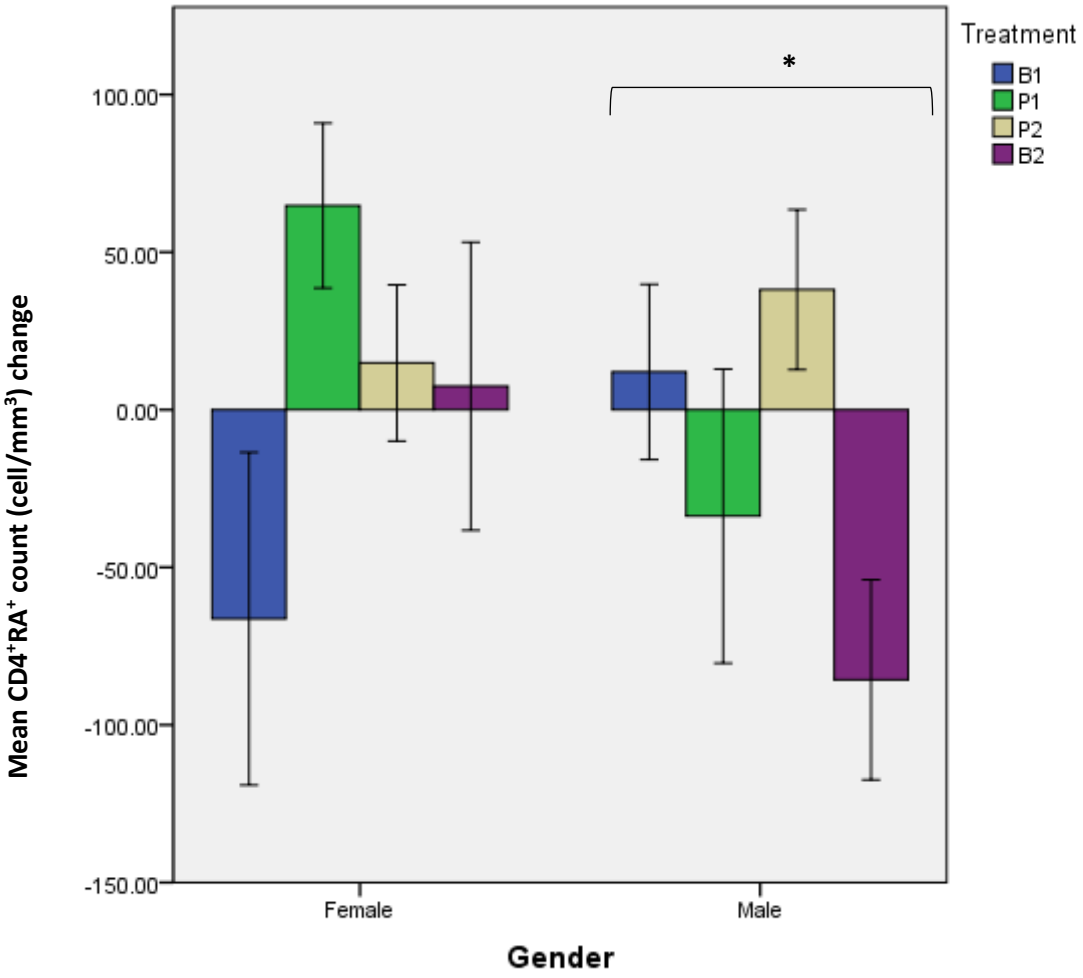

Figure 4.21. $CD4^+RA^+$ count (cells/mm^3) change observed in participants according to treatment consumed. Females: n = 17 for B1, P1, and B2; n = 15 for P2; males: n = 22 for B1, n = 25 for P1 and P2, n = 26 for B2. Both phases have been combined. * Significant at $P < 0.05$, as analysed by the Kruskal-Wallis test. Error bars: +/- 1 SD.

Table 4.24. Effect of the treatments on lymphocyte subset count in female participants.

	Group 1				Group 2			
Cell/mm^3	**B1 (N=17)**		**P1 (N=17)**		**B2 (N=17)**		**P2 (N=15)**	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
CD3$^+$	1708.8±389.5	1644.7±498.8	1727.5±411.5	1726.8±491.7	1691.5±447.7	1742.9±451.4	1834.2 (1260.6–1982.6)	1830.5 (1311.7–2071.6)
CD8	595.2±198.9	580.3±245.7	610.8 (405.4–752.4)	615.1 (526.4–752.4)	577.0±198.9	595.7±180.3	524.2 (421.1–706.9)	585.4 (452.9–722.8)
CD4$^+$	987.2±238.9	943.4±292.2	986.9 (800.1–1181.7)	1068.5 (916.5–1337.6)	1010.9±307.1	1034.4±313.8	1018.9 (814.6–1271.30)	873.1 (827.6–1271.3)
CD8$^+$RA$^+$	317.0 (204.3–488.6)	312.6 (172.7–377.3)	274.7±127.11	312.0±166.4	292.1 (172.8–441.5)	350.3 (186.0–420.6)	257.9 (187.9–293.6)	242.1 (199.1–349.2)
CD8$^+$RO$^+$	257.8±125.9	281.7±128.4	303.0 (210.0–428.0)	365.5 (251.8–464.7)	237.0 (215.2–297.2)	268.1 (232.2–333.4)	314.4 (136.1–442.4)	334.2 (144.3–455.8)
CD4$^+$RA$^+$	535.9±151.8	496.5±166.2	402.3 (303.8–607.3)	484.4 (388.0–635.9)	569.9 (344.0–658.9)	604.4 (427.35–709.4)	**465.6 (205.7–585.1)**	**492.2 (268.6–618.9)***
CD4$^+$RO$^+$	397.0 (281.4–587.5)	374.3 (247.4–624.7)	516.3±229.2	437.3±238.6	419.8 (277.2–512.5)	472.0 (353.0–664.1)	571.6 (336.0–707.1)	536.3 (383.6–713.6)
CD3$^-$16$^+$56	250.0 (152.5–315.4)	215.9 (117.4–405.3)	253.2 (138.8–341.8)	198.2 (128.1–259.9)	180.4 (163.7–272.7)	191.5 (131.6–329.2)	203.5 (128.9–350.3)	232.2 (111.6–285.7)
CD19$^+$	183.5 (154.9–254.9)	179.9 (119.7–263.2)	213.8±77.6	225.3±87.1	224.3±87.2	221.2±103.6	236.1±84.0	254.6±100.1
CD4/CD8	1.67 (1.42–1.98)	1.75 (1.24–2.25)	1.50 (1.33–2.26)	1.68 (1.34–2.26)	1.82 (1.47–2.03)	1.84 (1.41–2.01)	1.85 (1.37–2.05)	1.78 (1.47–2.09)
CD3/CD19	8.40 (7.20–11.08)	9.19 (6.75–11.29)	7.93 (6.64–10.64)	8.44 (5.98–9.47)	7.01 (5.93–9.03)	7.01 (5.69–9.20)	6.48 (5.5–9.10)	7.01 (5.70–11.44)

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by the paired-*t* test or the Wilcoxon test, depending on normality. Significance at *P < 0.05. Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name.

Table 4.25. Effect of the treatments on lymphocyte subset count in male participants

	Group 1				Group 2			
cell/mm^3	**B1 (N=22)**		**P1 (N=25)**		**B2 (N=26)**		**P2 (N=25)**	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
CD3$^+$	1712.1±550.8	1684.3±506.0	1640.3±553.9	1786.5±528.9	1690.4±489.5	1519.5±390.7	1402.0 (1261.0–1634.2)	1420.2 (1268.1–1707.8)
CD8$^+$	623.1±302.6	586.4±219.7	512.1 (435.8–678.5)	571.0 (458.2–826.2)	577.2±264.7	525.9±212.7	501.4 (356.1–614.9)	435.1 (364.0–688.6)
CD4$^+$	944.1±289.4	958.2±341.1	938.1 (736.8–1079.5)	1039.5 (736.8–1079.5)	965.8±316.2	858.3±267.3	780.0 (654.6–901.7)	850.2 (731.2–976.1)
CD8$^+$RA$^+$	327.6 (173.8–399.1)	266.1 (175.4–416.9)	286.5±133.2	310.9±121.6	274.8 (180.0–391.0)	233.3 (179.4–331.9)	220.1 (168.1–313.0)	262.5 (175.9–289.8)
CD8$^+$RO$^+$	297.5±121.9	294.8±143.4	250.1 (213.6–335.8)	293.8 (209.2–387.4)	266.9 (158.2–382.2)	244.3 (165.9–337.7)	233.1 (170.8–317.0)	251.4 (186.8–341.6)
CD4$^+$RA$^+$	456.9±212.8	468.9±260.0	459.7 (314.4–512.5)	507.0 (376.7–655.9)	**436.0 (364.3 – 559.7)**	**384.65 (270.1–520.0)***	347.9 (313.3–413.8)	412.6 (339.5–492.1)
CD4$^+$RO$^+$	429.7 (264.2–614.8)	470.2 (265.3–660.9)	419.2±172.0	478.5±212.4*	411.4 (313.9–542.1)	404.5 (326.5–455.7)	401.3 (236.5–477.1)	385.0 (294.1–544.0)
CD3$^-$16$^+$56	289.2 (177.9–578.0)	299.2 (158.7–362.3)	**219.7 (162.3–360.7)**	**267.6 (193.7–467.9)***	238.4 (156.2–339.0)	225.7 (162.1–362.8)	240.0 (137.3–285.0)	222.7 (185.6–282.9)
CD19$^+$	256.9 (161.9–334.6)	224.1 (168.5–347.4)	233.9±104.4	256.3±126.8	288.9±90.6	261.9±77.9	247.5±87.4	260.3±71.8
CD4/CD8	1.56 (1.31–1.92)	1.64 (1.34–1.88)	1.66 (1.29–1.95)	1.51 (1.37–1.82)	1.95 (1.27–2.28)	1.65 (1.41–2.18)	1.85 (1.28–2.20)	1.82 (1.31–2.22)
CD3/CD19	6.74 (5.41–7.61)	6.62 (5.06–9.38)	7.21 (5.77–8.91)	7.46 (5.16–9.50)	6.06 (4.48–7.43)	6.01 (4.39–7.56)	5.65 (5.11–7.20)	5.65 (5.11–7.28)

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by the paired-*t* test or the Wilcoxon test, depending on normality. Significance at *P < 0.05. Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name

Table 4.26. Effect of the treatments blood biochemistry in female participants.

	Group 1				Group 2			
	B1 (N=19)		P1 (N=19)		B2 (N=19)		P2 (N=17)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Glucose (mmol/L)	4.8 (4.4 – 5.2)	4.3 (4.0 – 4.6)	4.8 (4.6 – 5.3)	4.6 (4.0 – 4.7)	**4.8 (4.7 – 5.1)**	**4.4 (3.8 – 4.8)***	**4.8 (4.4 – 5.1)**	**4.4 (3.9 – 4.8)***
NEFA (mmol/L)	0.03±0.01	0.03±0.01	0.03 (0.02 – 0.03)	0.03 (0.02 – 0.03)	0.02 (0.02 – 0.03)	0.03 (0.02 – 0.03)	0.03 (0.02 – 0.03)	0.03 (0.02 – 0.03)
Insulin (mU/l)	77.1±43.7	160.6±125.6	57.4±39.7	103.8±123.8	98.4±79.0	61.4±58.9	64.4±33.0	80.7±77.6
Triglycerides (mmol/L)	3.4 (2.4 – 5.4)	4.7 (3.0 – 6.2)	4.0 (2.8 – 8.1)	3.4 (2.8 – 5.9)	4.3 (3.4 – 5.5)	3.6 (1.2 – 4.9)	4.5 (3.3 – 5.9)	4.5 (2.8 – 5.7)
Cholesterol (mmol/L)	8.9±1.9	8.9±1.31	9.2±2.0	8.6±1.7	**10.1±1.8**	**8.6±2.0***	10.2±2.2	9.5±2.3
VLDL-c (mmol/L)	0.7 (0.5 – 1.1)	0.9 (0.6 – 1.3)	0.8 (0.6 – 1.6)	0.7 (0.6 – 1.2)	0.8 (0.7 –1.1)	0.8 (0.6 – 1.0)	0.9 (0.7 – 1.2)	0.9 (0.6 – 1.1)
LDL-c (mmol/L)	5.1±1.3	5.0±1.0	5.4±1.4	4.9±1.1	**6.0±1.5**	**5.0±1.5***	6.0±1.9	5.7±1.8
HDL-c (mmol/L)	2.8±0.9	2.9±0.9	2.7 (2.1 – 3.4)	2.3 (2.2 – 3.2)	**2.9 (2.7 – 3.5)**	**2.5 (2.2 – 3.2)***	**3.3 (2.9 – 3.6)**	**2.9 (2.4 – 3.1)***
APOA (mmol/dL)	8.4 (7.3 – 9.3)	8.7 (7.6 – 9.7)	8.4±1.3	8.2±1.6	**8.0 (7.4 – 9.3)**	**7.9 (7.29 – 9.2)***	8.8 (7.7 – 9.5)	8.5 (8.2 – 9.2)
APOB (mmol/L)	4.4 (3.9 – 4.8)	4.3 (3.8 – 4.7)	4.5±0.9	4.3±0.9	4.1 (3.5 – 5.3)	4.0 (3.5 – 5.1)	4.2 (3.6 – 5.2)	4.7 (3.8 – 5.4)

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by paired-*t* test or Wilcoxon test, according to normality. Significance at *P < 0.05. NEFA: non-esterified fatty acids; VLDL-c: very low-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; APOA: apolipoprotein A; APOB: apolipoprotein B.

Table 4.27. Effect of the treatments blood biochemistry in male participants.

	Group 1				Group 2			
	B1 (N=23)		P1 (N=26)		B2 (N=26)		P2 (N=25)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Glucose (mmol/L)	5.0 (4.5 – 5.2)	4.9 (4.6 – 5.3)	5.0 (4.7 – 5.2)	5.0 (4.9 – 5.4)	**5.1 (4.5 – 5.3)**	**5.3 (5.0 – 5.6)***	5.1 (4.8 – 5.2)	5.1 (4.7 – 5.4)
NEFA (mmol/L)	0.03±0.01	0.03±0.01	0.03 (0.02 – 0.04)	0.03 (0.03 – 0.03)	**0.03 (0.02 – 0.03)**	**0.03 (0.02 – 0.04)***	**0.03 (0.02 – 0.03)**	**0.03 (0.02 – 0.04)***
Insulin (mU/l)	607.2±1906.3	248.1±717.8	189.1±380.2	88.9±143.9	106.6±140.9	97.4±169.5	149.5±285.7	126.5±317.7
Triglycerides (mmol/L)	5.2 (3.3 – 8.1)	5.9 (3.7)	5.7 (4.2 – 8.3)	5.9 (3.9 – 6.8)	6.2 (3.7 – 10.1)	4.3 (3.3 – 8.3)	4.2 (3.2 – 6.5)	5.1 (3.5 – 7.8)
Cholesterol (mmol/L)	9.1±1.4	171.0±29.5	167.7±25.8	174.0±24.7	172.3±37.0	179.9±26.6	168.6±41.2	180.0±33.9
VLDL-c (mmol/L)	1.1 (0.7 – 1.6)	1.2 (0.7 – 1.8)	1.1 (0.8 – 1.7)	1.2 (0.8 – 1.3)	1.3 (0.7 – 2.0)	0.9 (12.0 – 29.5)	**15.0 (0.7 – 1.3)**	**1.0 (0.7 – 1.6)***
LDL-c (mmol/L)	5.3±1.2	5.5±1.3	5.6±1.2	5.7±1.4	5.8±1.4	6.2±1.3	5.9±1.7	6.2±1.7
HDL-c (mmol/L)	2.5±0.6	2.6±0.7	2.3 (2.2 – 2.7)	2.7 (2.2 – 2.9)	2.4 (2.0 – 2.7)	2.4 (2.3 – 2.6)	2.3 (2.1 – 2.6)	2.5 (2.2 – 2.7)
APOA (mmol/L)	7.4 (4.8 – 11.1)	7.8 (6.8 – 8.5)	7.8±1.0	7.9±1.1	7.4 (6.9 – 7.9)	7.4 (6.9 – 8.0)	7.2 (6.8 – 8.2)	7.7 (7.2 – 8.2)
APOB (mmol/L)	4.3 (3.8 – 5.3)	4.3 (3.7 – 4.8)	4.5±0.9	4.2±1.0	4.6 (3.9 – 5.7)	4.6 (3.9 – 5.1)	**4.4 (3.8 – 5.5)**	**4.3 (3.7 – 5.1)***

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by paired-*t* test or Wilcoxon test, according to normality. Significance at *P < 0.05. NEFA: non-esterified fatty acids; VLDL-c: Very low-density lipoprotein cholesterol; LDL-c: Low-density lipoprotein cholesterol; HDL-c: High-density lipoprotein cholesterol; APOA: Apolipoprotein A; APOB: Apolipoprotein B.

Table 4.28. Effect of the treatments on adipokines, acute phase proteins and cytokines in female participants.

	Group 1				Group 2			
	B1 (N=17)		P1 (N=17)		B2 (N=17)		P2 (N=15)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Ghrelin (pg/ml)	**174.9±105.5**	**368.4±136.2****	**126.5 (71.4–267.5)**	**359.5 (280.3–459.9)****	**167.7 (97.9–341.5)**	**465.3 (328.3–643.9)****	**247.0±165.5**	**549.3±197.9****
Leptin (ng/ml)	10.89±7.34	13.27±8.02	**9.03±4.67**	**14.64±6.99****	**10.05±7.92**	**12.63±6.86****	**6.92±2.93**	**11.11±6.62****
Adiponectin (ng/ml)	6721±5216	6478±4771	6999±4825	6109±5283	5367 (3770–8407)	6545 (5458–7845)	7763±4200	6660±3059
Ceruloplasmin (mg/dl)	32.9±7.4	33.5±9.6	31.9±8.9	32.1±9.4	29.3±7.1	29.4±7.6	30.6±7.9	31.8±8.9
CRP (mg/dl)	1.46 (0.34–4.11)	1.30 (0.50–7.22)	3.03±3.49	3.17±4.00	0.54 (0.41–1.20)	0.39 (0.18–1.52)	1.68±1.95	1.86±2.53
IL-1β (pg/ml)	0.17 (0.03–0.45)	0.61 (0.28–3.18)	0.93 (0.38–1.57)	1.42 (0.33–2.08)	0.30 (0.17–0.59)	0.69 (0.37–0.85)	7.52±11.63	1.57±2.32
IL-2 (pg/ml)	16.0 (1)	10.32 (4.33–11.29)(5)	(0)	8.30±3.56 (4)	5.20 (1)	5.92 (4.33–8.06) (5)	66.61 (1)	8.45 (3.28–160.9)(4)
IL-4 (pg/ml)	0.16 (1)	1.77 (1)	0.17 (0.06–0.44)(4)	1.04 (0.27–2.75)(4)	0.32 (1)	0.75±0.43 (2)	0.24±0.07 (3)	0.85±0.15 (2)
IL-6 (pg/ml)	**5.18 (1.52–9.22)**	**4.73 (3.52–25.04)***	9.10±9.54	10.94±9.46	6.84 (2.48–15.97)	6.82 (4.43–14.71)	7.53 (1.79–21.03)	5.86 (3.52–11.72)
IL-8 (pg/ml)	15.5 (2.3–21.1)	11.7 (10.2–17.6)	**9.4 (1.8–21.4)**	**12.5 (6.7–26.2)***	14.0 (1.5–23.1)	10.6 (7.0–16.4)	16.7 (2.4–22.1)	12.1 (8.6–17.0)
IL-10 (pg/ml)	2.90 (1.01–10.86)	5.05 (2.42–9.55)	1.20 (0.24–11.99)	1.94 (1.19–3.50)	0.40 (0.07–0.88)	2.46 (1.30–38.46)	0.14 (0.02–5.85)	1.58 (1.12–1.88)
TNF-α (pg/ml)	2.04 (0.75–4.03)	1.48 (0.93–34.50)	1.22 (0.72–2.43)	1.04 (0.62–32.24)	1.18 (0.57–2.83)	0.93 (0.66–1.81)	3.12 (0.72–11.34)	2.26 (0.16–9.95)
VCAM-1 (ng/ml)	**113.4±18.0**	**130.2±16.1***	113.3±19.4	107.2±27.0	119.1±30.8	112.9±25.4	115.1±34.5	107.1±30.1
ICAM-1 (ng/ml)	93.0 (73.7–110.9)	93.2 (72.2–100.8)	86.2 (70.3–103.4)	93.5 (69.4–107.7)	77.9 (73.1–93.9)	89.5 (67.8–106.6)	82.7 (65.0–92.5)	85.5 (64.9–92.9)

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by the paired-*t* test or the Wilcoxon test, according to normality. Significance at *P < 0.05. CRP: C-reactive protein; GM CSF: granulocyte macrophage colony-stimulating factor; IL: interleukin; IFNγ: interferon gamma; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1; ICAM-1: Intracellular adhesion molecule 1. For IL2 and IL4, n stated next to value, in brackets.

Table 4.29. Effect of the treatments on adipokines, acute phase proteins and cytokines in male participants.

	Group 1				Group 2			
	B1 (N=22)		P1 (N=25)		B2 (N=26)		P2 (N=25)	
	Baseline	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**	**Baseline**	**Endpoint**
Ghrelin (pg/ml)	263.4±93.8	1362±5175	241.3 (183.4–368.4)	258.6 (197.4–333.0)	281.2 (188.1–365.5)	236.9 (172.0–303.3)	442.8±913.6	290.3±139.1
Leptin (ng/ml)	3.59±3.38	3.75±3.59	**2.69±1.91**	**3.47±2.32***	3.60±2.37	4.31±3.45	**3.25±2.02**	**3.99±2.42***
Adiponectin (ng/ml)	4230±3204	3859±2777	3967±1952	3597±1687	4057 (2778–5522)	4195 (2950–5157)	4728±2296	4101±2057
Ceruloplasmin (mg/dl)	25.2±4.5	25.9±7.4	24.6±3.8	24.2±4.2	28.0±13.2	24.3±4.7	24.8±4.5	24.2±3.8
CRP (mg/dl)	0.44 (0.19–2.10)	0.67 (0.23–2.83)	**1.22±1.64**	**1.76±1.98***	0.54 (0.18–1.44)	0.37 (0.15–1.62)	1.03±1.54	1.37±2.18
IL-1β (pg/ml)	6.13 (1.04–674.1)	0.97 (0.83–1.29)	39.43 (1.18–77.7)	0.96 (0.88–1.13)	0.67 (0.36–335.5)	1.09 (0.93–1.74)	70.1±147.0	37.7±167.3
IL-2 (pg/ml)	22.4 (3.5–39.3)	11.8 (2.4–44.0)	34.4±28.7	12.9±14.2	35.3 (10.6–63.9)	4.9 (2.0–39.9)	73.6 (12.1–440.3)	13.7 (3.5–51.9)
IL-4 (pg/ml)	28.6±83.6	35.3±75.0	2.36 (1.21–2.94)	5.77 (0.05–24.3)	7.43±9.21	2.98±5.02	5.61±6.65	6.13±7.94
IL-6 (pg/ml)	9.66 (5.06–19.9)	6.48 (4.51–8.90)	**9.06±9.53**	**5.68±3.72***	10.97 (3.05–19.4)	5.14 (3.05–11.6)	10.72 (5.76–18.6)	5.35 (3.73–9.98)
IL-8 (pg/ml)	**23.4 (12.1–29.9)**	**15.6 (8.5–19.2)***	16.5 (10.1–31.0)	12.9 (8.0–20.1)	16.1 (9.2–56.4)	11.0 (8.4–19.0)	18.9 (14.0–35.2)	12.1 (9.8–27.3)
IL-10 (pg/ml)	4.51 (1.15–55.48)	2.59 (0.65–35.9)	3.87 (0.80–24.2)	2.91 (0.50–15.6)	2.90 (0.47–12.2)	1.54 (1.02–2.78)	3.21 (0.52–30.5)	4.59 (1.81–23.5)
TNF-α (pg/ml)	4.16 (0.44–9.88)	3.85 (1.27–9.72)	1.69 (0.71–14.26)	3.30 (2.38–5.26)	10.00 (0.87–27.39)	2.23 (1.28–8.30)	**4.58 (1.63–7.22)**	**4.00 (2.22–7.20)***
VCAM-1 (ng/ml)	**120.7±31.2**	**112.0±32.9****	**116.9±31.0**	**106.8±25.2***	121.2±26.2	115.2±23.6	**129.7±37.3**	**106.2±32.0****
ICAM-1 (ng/ml)	99.8 (82.8–125.0)	92.1 (73.4–177.4)	103.7 (87.1–118.7)	96.7 (89.1–149.9)	96.7 (80.7–116.5)	84.4 (78.3–164.4)	101.5 (79.2–118.5)	86.2 (70.7–136.8)

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and endpoint values compared by the paired-*t* test or the Wilcoxon test, according to normality. Significance at *P < 0.05. CRP: C-reactive protein; GM CSF: granulocyte macrophage colony-stimulating factor; IL: interleukin; IFNγ: interferon gamma; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1; ICAM-1: Intracellular adhesion molecule 1.

4.4. Predictors of responsiveness to weight loss treatments

Figure 4.22 illustrates the relative body weight changed observed in all participants between baseline and the end of the intervention with the active drinks (B1 and B2).

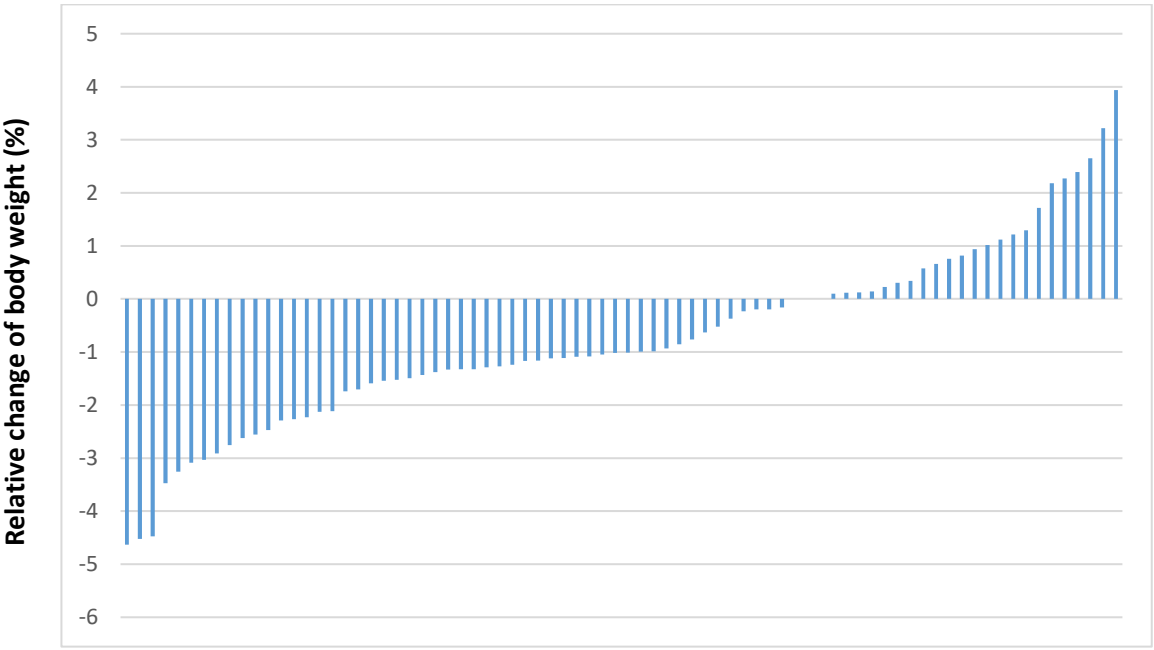

Figure 4.22. Individual relative body weight change (% initial weight) after treatments with either drink B1 or drink B2.

The analysis of correlations between relative body change and the variables studied identified compliance with treatment, baseline levels of triglycerides and VLDL-c counts of B lymphocytes ($CD19^+$) and GM-CSF as potential positive predictors of weight loss, while baseline BMI was a candidate negative predictor of weight loss (Table 4.30).

Table 4.30. Associations between relative body weight change and studied variables.

	ΔBody weight (%)			
	All		**Females**	**Males**
	r	**P**	**r**	**r**
Compliance	*-0.199*	*0.087*	**-0.422***	NS
BMI	*0.215*	*0.062*	NS	NS
Triglycerides	**-0.234**	**0.042**	NS	NS
VLDL-c	**-0.231**	**0.044**	NS	NS
B lymphocytes ($CD19^+$)	*-0.202*	*0.079*	NS	**-0.296***
GM-CSF	-0.083	0.637	**-0.422***	NS

Pearson's correlation; significance set at *P<0.05. BMI: body mass index; VLDL-c: very low density lipoprotein cholesterol; GM-CSF: Granulocyte-macrophage colony-stimulating factor.

When high and low responsiveness groups were compared, no significant differences were observed for the frequency distributions of gender (X^2 = 0.178, P = 0.458) or active drink consumed (B1 or B2) (X^2 = 0.025, P = 0.555) (Figures 4.23 and 4.24, respectively); therefore, both genders and drinks were pooled together for the rest of comparisons between high and low responsiveness.

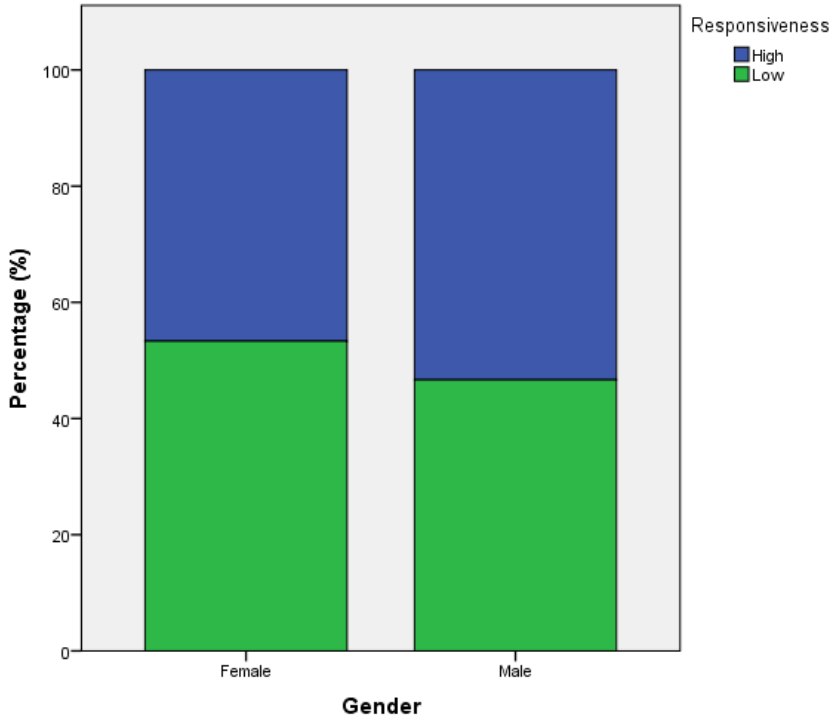

Figure 4.23. Distribution of high and low respondents in females and males (X^2 = 0.178, P = 0.458).

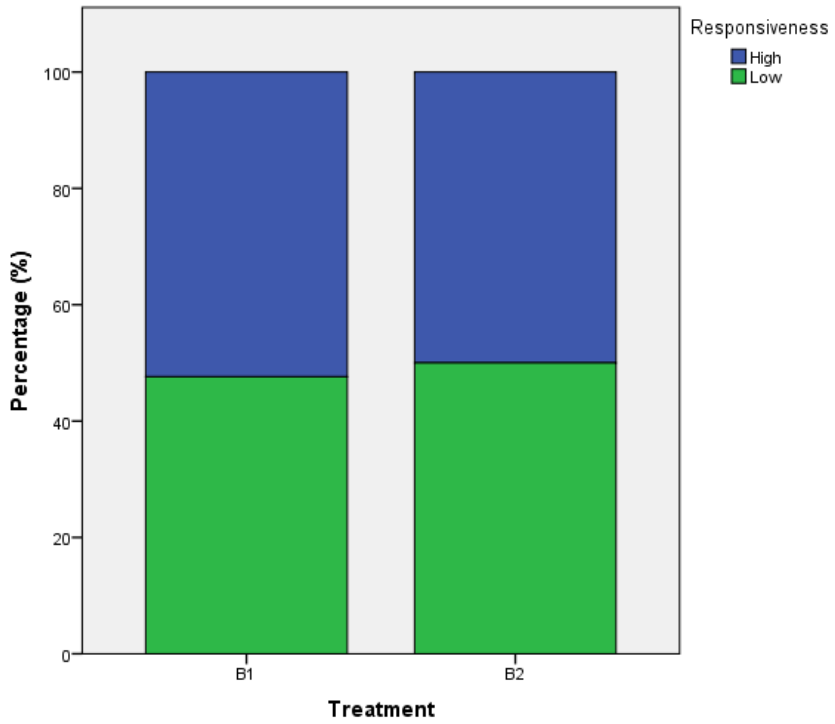

Figure 4.24. Distribution of high and low respondents in treatments B1 and B2 (X^2 = 0.025, P = 0.555).

There was no difference in compliance to the treatment between the high and the low responsive participants (96.9% v. 96.2%, respectively). When meal distribution patterns were compared, a lower frequency of midmorning snack consumption was associated with greater responsiveness to the treatment; there was also a trend for the low respondents to skip lunch more often than the high respondents (Table 4.31). No relationship was found between caffeine consumption and responsiveness (Table 4.32).

High respondents presented significantly lower baseline BMI and WHeR values (Table 4.33); trends towards higher MCHC and lower platelets counts (Table 4.34); lower T_C naive cell ($CD8^+RA^+$) counts, and as a result, a trend towards higher $CD8^+RO^+/CD8^+RA^+$ values (Tables 4.35 and 4.36); trends to lower LDL-c levels (Table 4.37), lower IL-8 and IL-10 values, and significantly lower IL-6 values (Table 4.38).

Table 4.31. Eating patterns in high and low respondents

		Responsiveness		
		High (%)	Low (%)	*P*
Breakfast	No	6.7	4.4	0.673
	Yes	44.4	44.4	
Midmorning	No	**31.1**	**11.1**	**0.005**
	Yes	**15.6**	**37.8**	
	Sometimes	**4.4**	**0**	
Lunch	No	*0*	*6.7*	*0.067*
	Yes	*51.1*	*42.2*	
Afternoon	No	33.3	24.4	0.403
	Yes	17.8	22.2	
	Sometimes	0	2.2	
Evening meal	No	2.2	2.2	0.613
	Yes	46.7	46.7	
	Sometimes	2.2	0	
Meals per day	≤3	13.2	7.9	0.547
	≥4	44.7	34.2	

Frequency of meals collected from the 3-day dietary records. High and low responsive groups compared by the Chi – squared; significance set at $P < 0.05$.

Table 4.32. Estimated caffeine consumption (mg/day) of high and low respondents.

	Responsiveness		
	High	**Low**	***P***
Total caffeine	107.2 (41.2 – 176.3)	91.0 (14.4 – 172.6)	0.447
Caffeine from tea and coffee	95.0 (17.3 – 106.1)	21.4 (4.0 – 152.6)	0.211
Caffeine from caffeinated and energy drinks	11.2 (6.7 – 41.2)	9.0 (1.6 – 24.5)	0.380

Data presented as median (Q1, Q3). High and low responsive groups compared by the Mann-Whitney test; significance set at P <0.05.

Table 4.33. Baseline anthropometric and body fat (%) values in high and low respondents.

	High responsiveness (N=22)	**Low responsiveness (N=22)**	***P***
Height (cm)	175 ± 0.1	170 ± 0.1	0.147
Body weight (kg)	84.9 ± 10.6	86.5 ± 15.2	0.673
BMI	**27.7 ± 2.3**	**29.6 ± 3.0**	**0.021**
Body fat (%)	24.2 (20.7 – 29.6)	26.7 (22.7 – 34.7)	0.302
WC (cm)	88.9 ±7.2	92.9 ± 11.2	0.154
WHR	0.83 ± 0.8	0.85 ± 0.09	0.452
WHeR	**0.50 (0.47 – 0.53)**	**0.54 (0.51 – 0.56)**	**0.006**

Data presented mean ± SD, or median (Q1, Q3); the Mann-Whitney or the independent-*t* tests were used, according to normality; significance set at P <0.05. WC: waist circumference; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio.

Table 4.34. Baseline values of red blood cell series in high and low respondents.

	High responsiveness (N=22)	**Low responsiveness (N=22)**	***P***
RBC (x10^{6}cells/mm^3)	4.86 ± 0.51	4.76 ± 0.42	0.488
Hb (g/dl)	14.5 ± 1.3	14.0 ± 1.2	0.153
Haematocrit (%)	43.8 ± 3.6	42.8 ± 3.6	0.322
MCV (fl)	90.5 ± 4.3	90.1 ± 4.5	0.716
MCH (pg)	30.0 ± 1.4	29.5 ± 1.5	0.253
MCHC (g/dl)	*33.1 ± 0.8*	*32.7 ± 0.8*	*0.088*
Anisocytosis Index (%)	11.9 (11.7 – 12.4)	12.4 (11.8 – 12.8)	0.207
Platelets (x10^3/mm^3)	*229.3 ± 44.3*	*255.1 ± 57.8*	*0.099*
MPV (fl)	9.5 ± 0.9	9.3 ± 0.9	0.436
Iron (mg/dl)	94.5 ± 37.0	88.8 ± 44.3	0.644
Ferritin (ng/dl)	87.0 (40.0 – 172.0)	66.0 (26.0 – 151.0)	0.276
Transferrin (mg/dl)	237.7 ± 46.2	59.4 ± 244.8	0.657

Data presented mean ± SD, or median (Q1, Q3); the Mann-Whitney or the independent-*t* tests were used, according to normality; significance set at P <0.05. RBC: red blood cells; Hb: haemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb concentration; MPV: mean platelet volume.

Table 4.35. Baseline values of white blood cell counts in high and low respondents.

	High responsiveness (N=22)	Low responsiveness (N=22)	*P*
White Blood Cells (x10^3/mm^3)	6.20 (5.20 – 7.10)	5.80 (5.10 – 6.90)	0.964
Neutrophil (x10^3/mm^3)	3.21 ± 0.82	3.40 ± 1.24	0.544
Lymphocyte (x10^3/mm^3)	2.10 (1.60 – 2.30)	2.10 (1.90 – 2.50)	0.864
Neutrophil/Lymphocyte ratio	1.50 (1.19 – 2.10)	1.63 (1.28 – 2.11)	0.708
Monocyte (x10^3/mm^3)	0.50 (0.30 – 0.60)	0.45 (0.40 – 0.60)	0.736
Eosinophil (x10^3/mm^3)	0.20 (0.10 – 0.20)	0.20 (0.10 – 0.30)	0.774
Basophil (x10^3/mm^3)	0.00 (0.00 – 0.10)	0.00 (0.00 – 0.00)	0.140

Data presented mean ± SD or median (Q1, Q3); independent *t*-test or Mann-Whitney test were used, according to normality; significance set at P <0.05.

Table 4.36. Baseline values of lymphocyte subsets in high and low respondents

Cell count (cell/mm^3)	High responsiveness (N=22)	Low responsiveness (N=22)	*P*
CD3$^+$	1586.0 ± 618.7	1555.3 ± 375.5	0.843
CD8$^+$	502.4 (338.4 – 576.5)	541.1 (402.0 – 624.3)	0.398
CD4$^+$	921.9 ± 345.2	881.9 ± 211.3	0.645
CD8$^+$RA$^+$	**140.1 (106.5 – 251.5)**	**260.1 (153.6 – 305.9)**	**0.027**
CD8$^+$RO$^+$	295.9 (220.2 – 386.2)	301.40 (219.9 – 364.6)	0.925
CD4$^+$RA$^+$	453.9 (377.3 – 662.1)	494.8 (380.7 – 585.7)	0.851
CD4$^+$RO$^+$	368.0 ± 235.4	351.1 ± 127.7	0.769
CD3$^-$16$^+$56	242.5 (155.7 – 334.2)	241.3 (168.0 – 300.6)	0.907
CD19$^+$	258.8 ± 105.4	230.6 ± 97.7	0.323
CD4/CD8	1.92 ± 0.76	1.79 ± 0.62	0.544
CD3/CD19	6.67 (4.48 – 8.25)	7.01 (5.68 – 8.44)	0.201
CD8RO/CD8RA	*1.99 (0.90 – 2.79)*	*1.12 (0.91 – 1.54)*	*0.071*
CD4RO/CD4RA	0.53 (0.36 – 1.06)	0.67 (0.43 – 0.98)	0.453

Data presented mean ± SD or median (Q1, Q3); independent *t*-test or Mann-Whitney test were used, according to normality; significance set at P < 0.05. Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name.

Table 4.37. Baseline values of blood biochemistry and iron levels in high and low respondents.

	High responsiveness (N=22)	Low responsiveness (N=22)	*P*
Glucose (mmol/L)	4.7 ± 0.8	4.8 ± 0.7	0.679
Insulin (mU/l)	98.3 (66.7 – 106.9) (14)	71.4 (52.6 – 126.5) (12)	0.471
HOMA	18.5 (12.3 – 23.0) (14)	15.4 (11.1 – 31.8) (12)	0.607
NEFA (mmol/L)	0.01 ± 0.01	0.02 ± 0.001	0.111
Triglycerides (mmol/L)	4.5 (1.3 – 7.6)	3.6 (3.2 – 5.9)	0.296
Cholesterol (mmol/L)	8.8 ± 1.6	9.3 ± 2.2	0.325
VLDL-c (mmol/L)	0.9 (0.7 – 1.5)	0.7 (0.6 – 1.2)	0.270
LDL-c (mmol/L)	*5.05 ± 1.9*	*5.8 ± 1.5*	*0.088*
HDL-c (mmol/L)	2.6 ± 0.7	2.7 ± 0.8	0.678
APOA (mmol/L)	8.0 (7.3 – 8.9)	8.1 (7.1 – 8.9)	0.820
APOB (mmol/L)	4.4 ± 1.1	4.6 ± 1.2	0.529

Data presented mean ± SD or median (Q1, Q3); independent *t*-test or Mann–Whitney test were used, according to normality; significance set at P < 0.05. NEFA: non-esterified fatty acids; VLDL-c: very low-density lipoprotein cholesterol; LDL-c: low-density lipoprotein cholesterol; HDL-c: high-density lipoprotein cholesterol; APOA: apolipoprotein A; APOB: apolipoprotein B. N for insulin and HOMA in brackets.

Table 4.38. Baseline values of adipokines, acute phase proteins, and cytokines in high and low respondents.

	High responsiveness (N=22)	Low responsiveness (N=22)	*P*
Ghrelin (pg/ml)	292.3 (196.4 – 365.4) (21)	268.4 (238.4 – 309.9)	0.923
Leptin (ng/ml)	2.9 (1.5 – 7.0)	6.0 (2.9 – 10.5)	0.159
Adiponectin (ng/ml)	4437.9 ± 2502.3	5481.0 ± 3231.3	0.238
Cerulopl (mg/dl)	25.1 (22.7 – 32.0)	27.5 (22.2 – 28.9)	0.925
CRP (mg/dl)	0.5 (0.1 – 1.1)	1.2 (0.2 – 2.7)	0.302
IL-1β (pg/ml)	3.5 (0.2 – 20.9) (7)	0.8 (0.5 – 6.1) (6)	0.731
IL-2 (pg/ml)	66.6 (0.9 – 147.4) (3)	140.2 (46.7 – 901.8) (6)	0.381
IL-4 (pg/ml)	0.7 (0.5 – 280.2) (3)	2.9 (1.0 – 16.9) (7)	0.667
IL-6 (pg/ml)	**10.5 (6.0 – 16.7)**	**18.2 (10.2 – 36.0)**	**0.021**
IL-8 (pg/ml)	*18.9 (12.1 – 23.1)*	*25.5 (16.1 – 63.9)*	*0.054*
IL-10 (pg/ml)	*1.4 (0.5 – 49.0) (11)*	*16.7 (4.4 – 161.2) (11)*	*0.088*
TNF-α (pg/ml)	3.4 (1.7 – 40.3) (16)	10.2 (1.3 – 35.6) (19)	0.545
VCAM-1 (ng/ml)	110.3 (95.2 – 132.8)	102.1 (86.2 – 131.5)	0.105
ICAM-1 (ng/ml)	91.7 (80.4 – 107.3)	91.1 (74.9 – 110.6)	0.622

Data presented mean ± SD or median (Q1, Q3); independent-*t* test or Mann-Whitney test used, according to normality; significance set at P < 0.05. CRP: C-reactive protein; IL: interleukin; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1. ICAM: intracellular adhesion molecule 1.

All analysis considered, the following variables were selected as likely candidates for predicting responsiveness to weight loss in multiple regression analysis: gender, type of functional drink consumed, compliance, triglycerides, VLDL-c and $CD19^+$ from the correlation analysis, and BMI, WHeR, $CD8^+RA^+$, and IL-6 from comparing high vs low responsiveness groups. A forced entry multiple regression analysis was conducted, where VLDL was excluded due to high collinearity with triglycerides, and WHeR was excluded due to high collinearity with BMI. The resulting model (Model 1) was a significant predictor of body weight change, explaining 20.8% variability, although not all predictors introduced were significant contributors (Table 4.39). A second analysis was then performed including only significant contributors, resulting in another model that was a significant predictor of body weight change, explaining 18.1% variability (Model 2).

Multiple regression analysis was then conducted on males and females separately. For female participants, the following variables were included as potential predictors: compliance and GM-CSF from the correlation analysis, and BMI, WHeR, $CD8^+RA^+$, and IL-6 from the comparisons high vs low responsiveness groups. A hierarchical analysis was performed including compliance as the most likely predictor, followed by GM-CSF, and then the rest of variables in a single block. Model 1 (compliance alone) was a significant predictor of body weight change, explaining 19.5% of the variability (Table 4.41). Model 2 (compliance and GM-CSF) was also a significant predictor, explaining 30% variability, but was not significantly better than Model 1 and the individual contributors lost strength. The model including all 6 variables was not a significant predictor of body weight change ($P = 0.325$) and revealed again high collinearity between BMI and WHeR. WHeR was therefore excluded from the analysis. Given that not all cases had values for GM-CSF levels, another regression analysis was conducted excluding this variable. The regression analysis revealed BMI and IL-6 as weak contributors, so that Models 3 and 4 were developed with compliance and naive T_C cells ($CD8^+RA^+$), explaining 17.8% and 25.2% of the variability (Table 4.40).

For male participants, the following variables were included in a hierarchical multiple regression analysis: $CD19^+$ from the correlations (first block), and BMI, WHeR, $CD8^+RA^+$, and IL6 from the comparisons high vs low responsiveness groups (second block). $CD19^+$ alone was a weak but significant predictor of body weight change, explaining 8.8% variability (Model 5), while the addition of the other variables resulted in a non-significant model ($P = 0.135$). A second analysis was conducted, excluding WHeR due to collinearity, and $CD8^+RA^+$ and IL-6 due to poor contributions. When BMI was added to the analysis, the model was not significantly better than Model 5 ($P = 0.133$ for the

comparison between models) and the individual contributions lost strength, but overall the model was still significantly good and explained 13.6% variability (Model 6) (Table 4.40).

Table 4.39. Multiple regression analysis of relative body weight change.

			Predictors			Final model	
Model	**N**	**Predictors**	**Coefficients**	**95% CI**	***P***	**R^2**	**P**
1	**75**	Gender	0.188	-0.614, 0.990	0.641	0.208	0.042
		Drink	-0.043	-0.815, 0.729	0.912		
		Compliance	-0.098	-0.197, 0.000	0.050		
		Triglycerides	-0.011	-0.018, -0.003	0.008		
		BMI	0.165	0.034, 0.296	0.014		
		$CD8^+RA^+$	0.000	-0.002, 0.003	0.669		
		IL6	0.000	0.000, 0.001	0.229		
		$CD19^+$	-0.002	-0.006, 0.003	0.471		
2	**75**	Compliance	-0.097	-0.191, -0.002	0.046	0.181	0.003
		Triglycerides	-0.010	-0.017, -0.004	0.004		
		BMI	0.157	0.034, 0.280	0.013		

Unstandardized-β coefficients, 95% confident intervals and individual P values are shown for each predictor included in the analysis.

Table 4.40. Multiple regression analysis of relative body weight change in females and males.

				Predictors			Final model	
Gender	**Model**	**N**	**Predictors**	**B**	**95% CI**	***P***	**R^2**	**P**
Females	1	30	Compliance	-0.204	-0.402, -0.005	0.045	0.195	0.045
	2	21	Compliance	-0.162	-0.360, 0.035	0.102	0.300	0.040
			GM-CSF	-0.001	-0.003, 0.000	0.117		
	3	30	Compliance	-0.239	-0.437, -0.040	0.020	0.178	0.020
	4	30	Compliance	-0.247	-0.441, -0.054	0.014	0.252	0.020
			$CD8^+RA^+$	-0.003	-0.007, 0.001	0.113		
Males	5	45	$CD19^+$	-0.005	-0.009, 0.000	0.049	0.088	0.049
	6	45	$CD19^+$	-0.004	-0.009, 0.000	0.061	0.136	0.047
			BMI	0.115	-0.037, 0.266	0.133		

Unstandardized-β coefficients (B), 95% confident intervals and individual P values are shown for each predictor included in the analysis. Significance set at $P < 0.05$. GM-CSF: Granulocyte-macrophage colony-stimulating factor.

5. Discussion

5.1 Relationship between obesity and early developmental factors

The aims of this study were to explore the relationships between indices of obesity and several biological factors (early development, dietary habits, markers of metabolic function and inflammation, and circulating immune cells); to investigate the effect of a polyphenol-based weight loss intervention on the indices of obesity and the parameters studied; to assess individual responsiveness to the intervention; and to determine if and what factors may be predictors of responsiveness.

My results support a potential protecting effect of breastfeeding against abdominal obesity in both male and female participants. The findings of my study showed a general trend for lower indices of obesity in those who were breastfed the first four months of their lives. The potential protecting effect of breastfeeding appeared to be more prominent in the female participants, with a significant reduction in female's waist circumference on those who were breastfed.

Various studies have suggested that breastfeeding during infancy confers protection against diseases; for example, breastfeeding is associated with reduced risk of a number of neonatal infections such as gastro-intestinal, diarrhoeal, and certain types of extra-intestinal infections (Jackson & Nazar, 2006). There is also evidence for a negative relationship between breastfeeding and obesity, showing increased obesity rates in non-breastfed populations and individuals (Poulton & Williams, 2001; Parson *et al*., 2003; Frye & Heinrich, 2003; Owen *et al*., 2005). The idea of the importance of infant nutrition on an individual's long-term adiposity was first suggested in the 1960s through the revolutionary work of McCance (1962). He showed that rats who were breastfed for longer as infants were larger as adults. This effect was not observed with nutritional intake after weaning, suggesting nutrition acts in the critical early post-natal window, which programs later body size (McCance, 1962; as reviewed by Singhal *et al*., 2007). Although it is noted that a variety of complex factors affect obesity, a 15% to 30% reduction in adolescent and adult obesity has been observed if any breastfeeding happened in infancy compared with no breastfeeding at all (Owen *et al*. 2005; Ip *et al*. 2007).

Dewey and co-workers (2003) proposed three possible explanations for the protective quality breastfeeding has against obesity: learned self-control, properties of the breast milk and/or metabolic programming, and residual confounding by parental attributes and/or the family environment. The authors discussed first that breastfeeding was able to instil the ability of self-control in the infant. Breastfeeding, compared to formula feeding, teaches the infant to control the amount of milk they wish

to consume, whereas bottle-fed infants may be encouraged to finish the bottle (Dewey & Lonnerdal, 1986; as reviewed by Dewey *et al*., 2003). Secondly, breast milk is believed to contain different hormones, including leptin (*Casabiell et al*., 1997). Leptin exerts a key role as a regulator of appetite and adiposity (Dewey *et al*., 2003). Adiposity is positively correlated with leptin, and under normal conditions (in the absence of leptin resistance), increased leptin levels will inhibit appetite. Although the adipose tissue is the primary producer of the hormone, leptin is also secreted by other tissues, such as the mammary epithelium (Smith-Kirwin *et al*., 1998) and is therefore naturally present within breast milk (Casabiell *et al*., 1997; as reviewed by Palou & Pico, 2009). Dewey and colleagues discussed that breastfeeding may affect leptin metabolism both in childhood and throughout later life. The concentration of leptin within human milk can vary significantly, and it is positively correlated with maternal plasma levels and adiposity (Houseknecht *et al*.,1997; Miralles *et al*., 2006; as reviewed by Palou & Pico, 2009). Thus, the infant's exposure to leptin during lactation is directly linked to the adiposity of the mother, suggesting that the positive effects of breastfeeding may be negated by maternal obesity.

In addition, breast milk has the ability of modulating metabolic programming. This has been suggested from the finding that infants who were formula fed had elevated insulin levels and prolonged insulin response compared to those who were breastfed (Lucus *et al*., 1981; Owen *et al*., 2006). Increased insulin levels are able to stimulate adiposity (Odeleye *et al*., 1997). The mechanism by which feeding style can alter insulin levels is through the infant's intake of protein; it was reported that infants fed by formula consumed 66% to 70% more protein than their breastfed counterparts and diets with increased protein content encourage insulin secretion(Heinig *et al*., 1993). In addition to this, a study conducted by Dorotsy and co-workers (2000) found a positive association between high protein intake in early life and obesity in childhood.

In my study we also observed a U-shape relationship between birth weight and indices of obesity, particularly in females. In addition, significant positive associations presented between birth weight and BMI, waist circumference and waist-to-height-ratio in males.

Positive correlations have been identified between birth weight and ensuing childhood and adult obesity (Rasmussen & Johansson, 1998; Celi *et al*, 2003; Rugholm *et al*, 2005; Monasta *et al*, 2010; as reviewed by Yu *et al*, 2011). A study conducted by Kahn and co-workers (2000) suggested the increased prevalence in elevated adult body weight observed in individuals who had a higher birth

weight may be due to an increased lean mass, instead of an increased amount of fat tissue. Their study found that adult BMI reflected increments in lean tissue more than increments in adipose tissue (Kahn *et al*, 2000). Therefore, Kahn and co-workers would indicate that increased birth weight is not an indicator of obesity but of greater body weight, which could explain elevated levels of BMI seen in the participants, as no relationship was identified in body fat (%).

In contrast, Schellong and colleagues (2012) demonstrated that a higher birth weight (4 kg and above) lead to a two-fold increased risk of obesity. They did so through a meta-analysis of data from western and westernized, developing countries from northern and southern America, Europe, Asia, and Australia, and the increased risk obtained was independent of confounding variables like geographic/ethnic origin, socio-economic status, or parental weight status. The study conducted by Schellong and colleagues did not discuss however the relationships or associations between reduced birth weights and increased prevalence of obesity.

A study by Sorensen and co-workers (1997), did present, similar to my study, a U-shaped association, showing higher prevalence of obesity for the lowest and highest birth weights. Labayen and co-workers (2008) showed that a lower body weight was linked to inadequate intrauterine conditions (*e.g*., excessive or insufficient energy supply, deficiencies or excess of specific nutrients, maternal stress, etc.) that lead to abnormal foetal development. Connections have been made between decreased infant birth weight and lower lean body mass and greater central obesity, using measurements such as waist-to-hip ratio or skinfold thickness in adults (Labayen *et al.*, 2008). This was further suggested in this present study which presented significant associations between decreased birth weight and elevated indices of abdominal obesity in both male and female participants.

Another explanation proposed between low birth weight and adult obesity is the development of the thrifty phenotype. The hypothesis proposes that pregnant women exposed to poor nutritional conditions can alter an unborn child development in such a way that it will be prepared for survival in an environment lacking in resources (Hales & Barker, 1992). Hale and Barker (1993) hypothesised that the in utero low sustenance resulted in the development of a thrifty phenotype in anticipation of a life with limited food (as reviewed by Beauchamp *et al*., 2015). Beauchamp and co-workers (2015) found that the mice whose mothers experienced undernourishment during their pregnancies had elevated levels of adiposity in adulthood, and when entered into a weight loss program presented a significantly reduced ability to lose weight. They also observed that those subjected to undernutrition

had decreased mitochondrial content in mixed fibre muscles and reduced respiration in mitochondria, which contributed to elevated adiposity.

5.2 Relationship between obesity and dietary factors

This study did not find a relationship between the indices of obesity and caffeine consumption in the participants. Over 55% of adults are reported to consume coffee or tea (Bleich *et al.*, 2009), and various epidemiological studies have reported relationships between tea and coffee consumption and reduced risk of type 2 diabetes and coronary heart diseases (Higdon *et al.*, 2006; Schneider & Serge, 2009; Balk *et al*., 2009). Greenberg and co-workers (2005) hypothesised that this protective nature of coffee and tea from type 2 diabetes might be linked to a reduction in obesity. Inconsistency appears, however, throughout the available literature. Various other studies found that coffee and tea consumption presented no relationship with obesity, as shown in my study (Egger *et al.*, 1999; Van Dam & Feskens, 2002; Rosengren *et al.*, 2004; Tuomilehto *et al.*, 2004; Bouchard *et al.*, 2010). The use of additives such as milk, sugar and sweeteners may reduce the anti-obesity qualities of tea and coffee (Balk *et al.*, 2009; Bouchard *et al.*, 2010), as research has presented positive associations between body weight gain and milk/dairy (Moore *et al*., 2008), sugar (Bray & Popkin, 2014) and even sweeteners (Feijo *et al*., 2013). We did not analyse these additives in this study, so a clear conclusion requires further analysis.

Another avenue investigated within this study was the relationship between meal distribution patterns and indices of obesity. There is a consensus that breakfast consumption can protect against overweight and obesity versus skipping breakfast (Berg *et al.*, 2009; Huang *et al.*, 2010; Casazza *et al.*, 2013; Brown *et al.*, 2013; Reeves *et al.*, 2013; Deshmukh-Taskar *et al.*, 2013; O'Neil *et al.*, 2014). One of the largest and most comprehensive meta-analysis was conducted by Brown and co-workers (2013), where they analysed 88 study groups and yielded a pooled odds ratio of 1.55 (95% CI: 1.46, 1.65) for the likelihood of being overweight/obese among breakfast skippers versus consumers (Brown *et al.*, 2013; as reviewed by Barr *et al.*, 2016). Other authors, on the contrary (Song *et al*., 2005; Grujic *et al.,* 2009; Lee *et al.*, 2016), found no significant relationship between breakfast consumption and BMI or prevalence of overweight/obesity. In the current study, we found no significant differences in the indices of obesity between participants who consumed breakfast and those who did not, although a trend was observed for lower obesity values in females who consumed breakfast. Song and co-workers (2005) found that female participants presented differences in the indices of obesity according to the type of breakfast they consumed. Those who ate a ready-to-eat

cereal for breakfast presented a lower odds ratio for being overweight or obese, compared to those who consumed other foods for breakfast. These findings could indicate that not just the act of eating breakfast, but the type of foods consumed could act as a protective factor against obesity, indicating that further study is needed to understand the effect of breakfast on obesity in this present study.

5.3. Relationship between obesity and blood cells

All indices of obesity (except WHR) were positively correlated with red blood cell counts in this studies participants. The current studies results are in agreement with other work (Vuong *et al*., 2014), and previous studies have linked increased erythropoiesis with the metabolic syndrome (Rangno *et al*., 2001; Mardi *et al*., 2005). A possible explanation for the relationship between red blood cell counts and obesity is an increase in insulin (Vuong *et* al., 2014), as insulin and insulin-like-growth factors enhance erythropoiesis (Ratajczak *et al.*, 1998; Miyagawa *et al.*, 2000). Other explanation may be sleep apnoea and/or obesity-related hypoxia (Graham *et al.*, 1980), leading to increased numbers of red blood cells to combat the oxygen deficit. In addition, adipokines such as leptin and adiponectin could be linked to erythrocyte metabolism (Tsuda & Nishio, 2004; Tsuda, 2006). Tsuda and Nishio (2004) presented data that clearly demonstrated that leptin had the ability to modulate the fluidity of the erythrocyte membrane. They suggested that this might be of considerable biological and clinical significance in determining rheological properties of the cell membranes. Leptin has elevated levels in those with increased adiposity (Ouchi *et al.*, 2011) and therefore this could link obesity to increased red blood cell counts.

On the other hand, a negative correlation was observed between BMI and abdominal obesity and the size and haemoglobin concentrations of red blood cells. One possible explanation for these associations may be iron deficiency (Vuong *et al.*, 2014), and actually a negative trend was observed between indices of obesity and iron levels in the present study. Tussing-Humphreys and co-workers (2012) found that obese individuals have elevated levels of hepcidin. Hepcidin is the main regulator of iron homeostasis, which in turn means it plays a critical part in the aetiology of several iron-related disorders. Hepcidin traps circulating iron in macrophages and liver cells, and decreases gut absorption; therefore, Tussing-Humphreys proposed that hepcidin might play an important role in iron depletion often observed in obesity. In this line, in the current study ferritin was found to be positively correlated with all indices of obesity. Ferritin is an intracellular protein that plays a critical role in the regulation of iron homeostasis, and has been shown to be a biomarker of elevated body iron stores (Cook *et al*., 1974). Ausk and Ioannou (2008) discussed that they found ferritin levels increased with

indices of obesity, and stated that obesity-related inflammation is expected to cause increased ferritin serum levels, as ferritin is a common acute phase reactant. Ausk and Ioannou theorised that serum ferritin might be used to reduce the oxidative stress by binding to free redox-active iron. Furthermore, obesity-related inflammation would induce hypoferremia, a biological response to infection (Means, 2004). Ausk and Ioannou therefore concluded that obesity association with changes in ferritin serum levels are linked to inflammation and not obesity-related anaemia. Therefore, the contradictory elevation in ferritin and negative trend between iron and obesity could be related to different processes. Various studies have also indicated a link between elevated body iron stores and metabolic syndrome, and elevated serum ferritin levels have been significantly associated with visceral and subcutaneous fat stores (Iwasaki *et al.*, 2005) and presented as a potential predictor of type 2 diabetes (Bao *et al*., 2012; Zhoa *et al*., 2012; Kunutsor *et al*., 2013). Elevated ferritin levels have also been linked to central adiposity (Gillum, 2001), in agreement with this current study's findings.

We also observed increased platelet counts with all indices of obesity (except WHR). Elevated platelet counts have been associated with insulin resistance and cardiovascular disease (Thaulow *et al.*, 1991; Taniguchi *et al.*, 2003). The participants within this current study did not present insulin resistance or cardiovascular disease; however, as previously discussed elevated adiposity presented in this study is linked to the development of metabolic syndrome-related disorders. Coban and co-workers (2005) found that obese participants presented greater mean platelet volume in comparison to non-obese controls. Platelet counts and platelet activation are associated to a person's state of chronic inflammation, and as obesity is a form of chronic inflammation, this would explain the association between platelets counts and indices of obesity (Yudkin *et al*., 2000).

Elevated counts of white blood cells were observed when all tested indices of obesity were raised (except body fat %). In agreement with the current study's findings, Fisch and co-workers (1975), in a study upon almost 15,000 healthy women, presented that obesity was associated with higher white blood cell counts. The makeup of inflammation and metabolic syndrome and its associated ailments have all been connected to elevated white blood cell counts (Nagasawa *et al*., 2004; Dehghani *et al*., 2016). Various studies have demonstrated a chronic inflammation status in individuals with elevated indices of obesity (Stofkova, 2009; Karalis *et al*., 2009; Iyer *et al*., 2010). This increase is believed to be due to the development of metabolic syndrome (Fadini *et al*., 2012). The increase in the number of white blood cells in participants with elevated indices of obesity within the

current study is similar to the increased measures of inflammation observed in other studies (Vallianou *et al*., 2010, Johnson *et al*., 2012).

Neutrophil counts presented significant positive correlations with all indices of obesity. This increase may be explained by a variety of chemo-attractants for neutrophils such as CXCL8 and CCL5, which are increased in obese individuals (Trellakis *et al*., 2012). Further to this, leptin is known to promote neutrophil chemotaxis and is presumed to be a survival cytokine for neutrophils (Fernandez-Riejos *et al.*, 2010). Neutrophil numbers are also associated with the formation of atheromatous plaque (Avanzas *et al*., 2004). Buyukkaya and co-workers (2012) found elevated numbers of neutrophils in individuals with metabolic syndrome, with the number of neutrophils increasing as metabolic syndrome became more severe. The elevated numbers of neutrophils seen in the current study may therefore indicate an early onset of metabolic syndrome.

In the current study, significant positive correlations were identified as well between monocyte counts and BMI and WHeR. This association has been observed in other studies (Kullo *et al*., 2002; Ilavska *et al*., 2012), and might be explained by increased secretion of monocyte chemoattractant protein (MCP)-1 by the elevated number of pre-adipocytes and adipocytes in obesity (Gerhardt *et al.*, 2001). Other studies, however, did not fully agree with the current study's findings; for example, Rogacev *et al*., (2010) looked into $CD16^+$ Monocytes and found no significant relationship/difference. Nevertheless, Rogacev and co-workers agreed that there was an association, and that obesity should not be discounted as a potential mediator of macrophage infiltration.

In contrast to neutrophils and monocytes, lymphocytes counts in the current study presented a negative correlation with body fat %. Perez de Heredia and co-workers (2015) found higher neutrophil/lymphocyte ratio association with increasing BMI, as seen within this present study. At the same time, there was a significant positive association between lymphocyte counts and WHR. This finding is more in line with other studies who found elevated lymphocyte counts and obesity (Marti *et al*., 2001).

WHR also presented positive associations with T mature cells ($CD3^+$), T cytotoxic ($CD8^+$), memory T cytotoxic $CD8^+RO^+$, and memory T helper $CD4^+RO^+$ cells presented positive correlations with BMI. Obese participants had significantly higher T/B lymphocyte ($CD3^+/CD19^+$) ratio than overweight ones. This is in agreement with other studies which found higher $CD3^+$ and $CD4^+$ cell counts with increasing BMI (Perez de Heredia *et al.*, 2015).

5.4 Relationship between obesity and serum and plasma variables

Obesity and abdominal obesity were positively associated with glucose, HOMA index, triglycerides, and VLDL, while total cholesterol was associated with abdominal obesity, in agreement with the most of literature (Kuksis *et al.*, 1991; Jones, 2013). Insulin resistance, disruption to lipoprotein metabolism, and increased serum triglyceride levels are common features in subjects with elevated indices of obesity and abdominal obesity (Despres *et al.,* 1990; Chan *et al*., 2002; Katsuki *et al*., 2003). It has been reported that elevated levels of triglycerides and glucose when fasting are useful indicators of insulin resistance (Simental-Mendía *et al*., 2008).

BMI also was significantly correlated with levels of leptin, cerpuloplasmin, CRP and ICAM-1. Pathophysiological mechanisms that link increased adiposity and elevated levels of CPR have been documented (Bastard *et al*., 2006; Ferrante, 2007; Rocha & Lippy, 2009; Brooks *et al*., 2010); increased adiposity leads to elevated CRP levels because adipose tissue is an active endocrine organ with the ability to release a range of cytokines and hormones (Van Gaal *et al*., 2006). The accumulation of free fatty acids caused by activated pro-inflammatory serine kinase cascades, for example IkB kinase and c-Jun-N-terminal kinase, leads to secretion of cytokines such as IL-6, which stimulates the synthesis of CRP (Rocha & Lippy, 2009). The increases in the levels of ceruloplasmin seen with elevated levels of obesity are in line with the findings of another study which found that adipose tissue of obese subjects can result in the overexpression of ceruloplasmin (Arner *et al*., 2014). Nishimura and co-workers (2008) how the endothelial cells within adipose tissue express higher levels of adhesion molecules, which would explain positive correlation.

5.5 Effect of the treatments

The current study used a selection of four treatments within this obesity intervention programme. Two of these tested treatments were active drinks (B1 and B2) and two placebos (P1 and P2). Only treatment B2 presented a significant reduction in female BMI, which was not observed in the placebo. In contrast, Nagao and co-workers (2005) presented significant reductions in body weight, BMI, waist circumference and body fat mass in their participants who were administer green tea extract. In comparison, it would appear that the treatment given in the current study was not successful in improving overweight.

Treatment B1 presented a significant increase in mean corpuscular Hb concentration in female participants. Vuong and co-workers (2014), as stated previously, demonstrated a link between obesity and reduced red blood cell size and mean corpuscular Hb concentrations. The improvement of the haemoglobin status in this studies female participants could also be due to reductions in adiposity levels, as seen in markers of abdominal obesity like waist and WHeR, although similar reductions were obtained with the placebo as well. In line with this theory, treatment B2 presented significant increases in haemoglobin and mean corpuscular Hb in males (but again similar increases were observed with the placebo).

Treatment B2 showed also significant reductions in total white blood cells and neutrophil counts. Even if we cannot ascertain that these reductions were linked to an improvement in indices of obesity, they could be a positive outcome, as higher white blood cell counts have been associated with higher risk of developing metabolic syndrome (Odagiri et al., 2011), and neutrophil counts have been connected with obesity (Trellakis *et al*., 2012). In addition to this, B2 presented a significant reduction of $CD4^+RA^+$ levels in males. Again, there is little research to date on the role of specific lymphocyte subsets on obesity, but a recent study suggested that $CD4^+$ can confer “obese memory”, and stimulate weight regain in rodents (Zou *et al*., 2017).

In relation to blood biochemistry, treatment B2 presented significant decreases in total cholesterol, LDL, and apoA1 in the female subjects, whereas it presented significant increases in glucose and NEFA in the male subjects. On the one hand, this may indicate that treatment B2 had a beneficial effect upon female participants’ lipid profile, and suggest higher lipolytic activity in male participants (as indicated by the rise in NEFA). On the other hand, these changes may indicate that

participants changed their lifestyle habits (e.g. diet, physical activity levels) during this trial, indicating that future research will need to investigate lifestyle throughout the intervention.

Significant increases were observed for ghrelin and VCAM-1, and reductions for IL-6 in female participants under treatment B1. The reduction in IL-6 could represent an improvement in the participants' immune status. Similarly to what was observed in immune cell counts, changes in IL-6 may not be a direct consequence of reductions in adiposity, but an indication of other beneficial effects of the polyphenol-rich treatments upon markers of inflammation. In addition to this, treatment B1 presented significant decreases in IL-8 and VCAM-1 in males, further supporting that participants' immune status improved.

5.6 Predictors of responsiveness to weight loss treatments

Successfulness of weight loss interventions is highly variable. Therefore, the identification of predictors of success is an important area of research. However, prediction of responsiveness to weight loss treatments have been demonstrated in literature to not be easily obtainable. We attempted to identify potential predictors of weight loss in the current studies participants in the hope of expanding the understanding of the great variability observed in the outcomes of most weight loss interventions. We used ΔBody weight (%) as the classifying criterion for success of intervention, because body weight is a key indicator of most weight loss treatments success. There was great variability in the response to the weight loss intervention in the current study (from -4.63% to +3.93% initial body weight).

In the search for a model to predict the response to a weight loss treatment, Wadden and colleagues (1992) presented that baseline body weight ($r = 0.27$, $P < 0.05$), weight loss in the first month ($r = 0.44$, $P < 0.001$) and compliance ($r = 0.37$, $P < 0.01$) were the most effectively correlated with weight loss after a low calorie diet or a mix of behavioural treatments over a 4-month period. Hansen and co-workers (2001) explained 8% of the variation observed in their study by the combination of baseline body weight, treatment group and age, while Leibbrand & Fichter (2002) found that baseline BMI was the only potential predictor to have a significant result at the 18-month follow up assessment ($R^2 = 0.07$, $P < 0.01$). It needs to be noted that although all these studies presented baseline BMI/body weight as significant predictors of weight loss, the variability explained by these factors was very low, being only 7% for BMI in Leibbrand & Fichter's, and less than 8% for

body weight in Hansen and co-workers'. This indicates the necessary contribution of other factors to explain why different individuals respond differently to weight loss treatments.

In the current study, the analysis of correlations between relative body weight change and baseline values of the studied variables showed that BMI was potentially a negative predictor of body weight loss, while compliance, triglycerides, VLDL-c, B lymphocytes ($CD19^{+}$) and GM-CSF were potentially positive predictors. These results present a contradiction, BMI, triglycerides and VLDL-c are generally associated (Mittendorfer *et al*., 2016). Mittendorfer and co-workers (2016); found that regulation of plasma VLDL-c and triglycerides concentrations is complex and influenced by a multitude if metabolic factors. Many individuals with obesity present normal plasma VLDL-c and triglyceride concentration (Mittendorfer *et al*., 2016).

In addition to this, participants were split into three groups (high responsiveness, with a loss of more than 1.5% of initial body weight; low responsiveness, with any increase in body weight; and those who could be classed as maintainers, with relative body weight change between -1.49% and 0%). The maintainers were excluded from the statistical analysis, and the high respondents and low respondents were compared to identify any differences in their baseline values. In contrast to the initial hypothesis, I did not find a significant difference in caffeine consumption between groups. Previous studies found that caffeine consumption demonstrated increased thermogenesis and therefore energy expenditure in the short term, which would contradict the current studies results; however, greater weight loss was not found over extended periods in comparison to the placebo (Pasman *et al.*, 1997; Lopez-Garcia *et al.*, 2006; Westerterp-Plantenga, 2010). This is believed to be due to the regular caffeine consumer becoming habituated to caffeine and losing sensitivity to caffeine (Westerterp-Plantenga, 2005). This would then explain the lack of significant differences between the current studies's high and low respondents according to their caffeine consumption. In contrast, Lopez-Garcia and co-workers (2006) found that long term high caffeine consumption reduced weight gain in comparison to subjects who consumed reduced amounts of caffeine, and this was explained through caffeine's influence on energy expenditure and intake. This combination of effects discussed within these two studies may be the reason for the lack of significant influence of caffeine consumption demonstrated within this present study.

High and low respondents presented no significant differences in meal distribution patterns, except for a lower frequency of midmorning snacking in high respondents. In contrast to the current study, other studies have shown inverse relationships between eating frequency and body fat

percentage and BMI in both lean and obese adults (Fabry *et al.*, 1964; Ma *et al.*, 2003). An US study found that adults consuming 4 or more meals per day presented a lower risk of obesity in comparison to those who ate three or less time per day (Ma *et al.*, 2003; as reviewed by Kulovitz *et al.*, 2014). Ohkawara and colleagues (2013) on the other hand found that there were no advantages of consuming smaller, more frequent meals on metabolism and appetite, and further suggested that it might have adverse effects on hunger and satiety. The disagreement in relation to the influence of meal frequency on weight loss leads to the need for future research.

Baseline BMI and WHeR were significantly lower in high respondents, and body weight, body fat percentage, waist circumference, and WHR presented trends in the same direction. Furthermore, a borderline positive correlation was observed between BMI and ∆Body weight (%). Therefore, baseline BMI appeared as a negative predictor of weight loss in the current study. Initial body weight and BMI have been previously identified as useful pre-treatment predictors of weight loss success (Wadden *et al*., 1992; Hansen *et al*., 2001; Leibbrand & Fichter, 2002; Vogels *et al.*, 2005; Hainer *et al.*, 2008) in calorie-controlled treatments. These studies reported that subjects with higher body weight lost more weight, as would be expected under reduced calorie conditions. However, as the current study was not calorie controlled, rather based on a supplement, it is interesting to see that those with reduced indices of obesity showed increased responsiveness. However, there appears to be very little literature regarding the relationship between responsiveness in a supplement weight loss treatment and indices of obesity. This clearly demonstrates the need for future investigation.

No significant differences were in either baseline red blood cell series or white blood cell counts between high and low respondents. However, it is worth noting that a trend appeared of decreased haemoglobin concentration in red blood cells and elevated platelet counts in participants who showed low responsiveness to the treatment. This may be explained by heightened indices of obesity and iron deficiency as previously discussed.

The lymphocyte subset $CD8^{+}RA^{+}$ was the only immune cell type studied to present a significantly different value between high and low respondents, with the latter showing increased counts. The literature does not give a clear answer to why this had happened. Why would low respondents have high values of naïve cytotoxic T cells, could it be suggested that an activated immune system helps with losing weight? It is not known, and further research in to the topic would be need. In addition, B lymphocytes count was a positive predictor of body weight loss. This is in contrast to other studies that have demonstrated impaired B cell capacity in obesity (Frasca *et al*., 2017), as

obese individuals fewer antibodies after vaccination (Karlsson *et al.*, 2016). It is not clear as to the reason for this relationship and clearly indicates the need for future studies.

Triglycerides and VLDL were also positive predictors of body weight loss, according to correlation analysis. Stroeve and co-workers (2016) concluded that 57% of the variation of weight loss observed between people undertaking the same treatment can be predicted by baseline plasma metabolic signature. In their study, the total level of triglycerides was negatively correlated with relative BMI change (Stroeve *et al.*, 2016), in concordant with the current study's findings.

Low respondent participants presented significantly elevated baseline IL-6 values, while GM-CSF on the contrary appeared as a positive predictor of weight loss in females. IL-6 secretion has been associated with BMI (Mohamed-Ali *et al.*, 1997). It has been estimated that 30% of all circulating IL-6 is derived from white adipose tissue (Fain *et al.*, 2004), and it has been observed that subcutaneous adipose tissue is responsible for the expression of a large amount of IL-6 (Fain *et al.*, 2004). IL-6 is a pro-inflammatory cytokine that promotes neutrophil and T helper 17 cell differentiation while blocking regulatory T cell (Treg) differentiation (Covarrubias *et al.*, 2014). Therefore, elevated values of IL-6 can indicate low grade inflammation, which is a precursor to metabolic syndrome. It has been shown that individuals with metabolic syndrome show reduced response to weight-management treatments compared to individuals without (Pi-Sunyer, 2005). However, it should be noted that this contradicts the findings that triglycerides acted as positive predictor. Triglycerides are a key feature of metabolic syndrome (Eckel *et al*., 2005) and therefore, if metabolic syndrome reduced an individual's ability to lose weight triglycerides would not be a positive predictor. The current literature does not give a clear explanation, and this indicates that further study is required.

According to these observations, we obtained a multiple regression model on our study sample including gender, type of drink consumed, compliance, BMI, triglycerides, B lymphocytes ($CD19^{+}$), T cytotoxic naive cells ($CD8^{+}RA^{+}$) and IL-6 as predictors. The model was a significant predictor of relative body weight change, explaining 20.8% of the variability, but it also showed that gender, type of drink, immune cells and IL-6 were not significant contributors. Therefore, a second model was developed including compliance, triglycerides, and BMI, which explained 18.1% of the variability and was a significantly good predictor of relative body weight change.

Robertson and co-workers (2016) found that females and males respond differently to a variety of weight loss interventions. Therefore, although gender wasn't identified as a significant predictor in the current studies regression analysis, we decided to also build models separately according to gender, and by doing this we found that there were different significant predictors for males and females.

In females, a regression model based on compliance alone was a significant predictor of body weight change, explaining 17.8% of the variability. Adding naïve T cytotoxic cells ($CD8^+RA^+$) explained 25.2% of the variability, but this model was not significantly better than compliance alone. In a subgroup of participants with valid values for GM-CSF, compliance and the chemokine could explain 30% variability, although again the model was not significantly better than compliance alone (which explained 19.5% variability in that subset). In male participants, $CD19^+$ alone explained 8.8% variability, and when BMI was added to the analysis, it explained 13.6% variability.

The current studies models explain a greater percentage of variability that previous studies, such has Leibbrand and Fichter (2002), who explained 7% of variability in treatment success based on baseline BMI, and Hansan and co-workers (2001), who explained 8% of variation by baseline body weight, treatment group and age. Handjieva-Darlenska and co-workers (2010) reported that the power of their regression analysis was significantly increased from 42% to 50.9% when the participants' first week's weight loss was added to the model. This could therefore be an interesting future model to follow, and an interesting early check to analyse whether the chosen weight loss program is suited to the patient.

5.7. Limitations, strengths and future work

The current studies study contributed to broaden the approach on identification of possible indicators of responsiveness to weight loss. The ability to predict weight loss allows a more specific intervention for the individual.

A strength of the current study was that it tested a far greater variety of biomarkers than previous studies. We explored the relationship between many metabolic biomarkers, and immune makers that have not been previously tested for their predictive potential in weight loss success.

The present study also has several limitations that have been discussed throughout and should be addressed. Firstly, a larger sample size would have increased the statically power, especially for immune status indicators, such as cytokine levels. Higher statistical power could have helped discriminate small differences in the values of serum biomarkers, and between pre- and post-treatment changes.

A limitation of this study is that the accuracy and reproducibility of the Tanita scale used to determine body fat % were not recorded at the time of data collection, and therefore are not available to me. The model used is mainly intended for domestic use, and as such, I can assume that its accuracy would be lower than that of a professional one. This would result in a potentially higher intra-individual variability of body weight and fat measurements, and in consequence a reduced statistical capacity to discriminate changes due to the intervention.

Another limitation of this study was the lack of analysis of the dietary and exercise habits of the participants. A more in depth analysis of energy intake and expenditure could have contributed to explain the differences observed in participants' responsiveness to the weight loss treatment. In addition, detailed information on specific nutrients, such as saturated and total fat or sugar, could help explain the variations in the levels of, cholesterol, triglycerides and glucose observed in participants in relation to the indices of obesity. This was not conducted due to the food diaries requiring knowledge of the Spanish language and food culture to be analysed, which implies considerable time and the participation of additional researchers. This being a master's project did not allow the time or resources to overcome this limitation.

Future works could involve the study of the influence of the identified potential predictors under different weight loss treatments, such as calorie-controlled diets, dietary supplements, or pharmaceutical. This may indicate whether certain types of treatment are more suited to individuals with specific features, thus creating a more personalised treatment plan, which will produce better results.

6. Conclusions

The current study supports a significant relationship between early developmental factors and obesity. The results agree with a potential protecting effect of breastfeeding for at least the first four months of life against adult obesity. We also observed a U-shape relationship between birth weight and obesity in females, and positive linear associations between birth weight and obesity in males.

The current study also identified reduced markers of haemoglobin status with obesity, supporting a relationship between obesity and anaemia, and significantly elevated levels of ferritin with obesity suggesting obesity related inflammation.

White blood cells presented significant elevations with higher indices of obesity (except body fat %) further indicating obesity-related inflammation.

Indicators of the onset of metabolic syndrome were also observed, with significant positive correlations between indices of obesity and glucose, triglycerides and cholesterol.

Overall, the intervention was not successful in reducing obesity in the sample, although treatment B2 appeared to effective in improving health markers, significantly reducing BMI, total white blood cells, neutrophil counts and $CD4^{+}RA^{+}$, and increasing haemoglobin and NEFA.

Compliance, triglycerides, VLDL, GM-CSF and B lymphocytes ($CD19^{+}$) were positive predictors and BMI was a negative predictor of weight loss. In addition, high respondents presented lower baseline BMI, WHeR, $CD8^{+}RA^{+}$ and IL-6. The regression analysis revealed compliance, triglycerides, and BMI constituted a good predictive model, which explained 18.1% of the variability.

Research in this field will allow a more personal and streamlined approach to weight loss and contribute to manage the current obesity epidemic.

7. Acknowledgements

I would like to thank Dr Fatima Perez De Heredia Benedicte, Prof Keith George, and Prof Ascension Marcos for the intellectual support and guidance that they have offered. I would also like of thank the PRONAOS project for the data used within this study and the financial aid that I received for printing and research by Liverpool John Moores University. As well as this, thanks would like to be given to the people who participated in this study.

8. References

Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B. (1996). Appetite-suppressant drugs and the risk of primary pulmonary hypertension. New England Journal of Medicine. 335: 609 – 616.

Akobeng A, Heller R. (2006). Assessing the population impact of low rates of breastfeeding on asthma, coeliac disease and obesity: the use of a new statistical method. Archive of Disease in Childhood. 92: 483–485.

Alberti K, Eckel R, Grundy S, Zimmet P, Cleeman J, Donato K, *et al*. (2009). Harmonizing the metabolic syndrome: a joint interim statement of the international diabetes federation task force on epidemiology and prevention; national heart, lung, and blood institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation. 120(16): 1640–1645.

Ashwell M, Hsieh S. (2005). Six reasons why the waist-to-height ratio is a rapid and effective global indicator for health risks of obesity and how its use could simplify the international public health message on obesity. International Journal of Food Sciences and Nutrition. 56: 303–307.

Ausk K, Ioannou G. (2008). Is obesity associated with anemia of chronic disease? A population-based study. Obesity Society. 16(10): 2356-2361.

Avanzas P, Arroyo-Espliguero R, Cosin-Sales J. (2004). Markers of inflammation and multiple complex stenosis (pancoronary plaque vulnerability) in patients with non-ST segment elevation acute coronary syndromes. Heart. 90(8): 847-852.

Avenell A, Broom J, Brown T, Poobalan A, Aucott L, Stearns S, Smith W, Jung R, Campbell M, Grant A. (2004). Systematic review of the long-term effects and economic consequences of treatments for obesity and implications for health improvement. Health Technology Assess. 8: 1-182

Aydin S, Aydin S, Ozkan Y, Kumru S. (2006). Ghrelin is present in human colostrum, transitional and mature milk. Peptides. 27(4): 878-882.

Baker J, Michaelsen K, Rasmussen K, Thorkild I. (2004). Maternal pre-pregnant body mass index, duration of breastfeeding, and timing of complementary food introduction are associated with infant weight gain. American Journal of Clinical Nutrition. 80: 1579–1588.

Balk L, Hoekstra T, Twisk J. (2009). Relationship between long-term coffee consumption and components of the metabolic syndrome: the Amsterdam Growth And Health Longitudinal Study. European Journal of Epidemiology. 24: 203–209.

Bann D, Hire D, Manini T, Cooper R, Botoseneanu A, McDermott MM, Pahor M, Glynn NW, Fielding R, King AC, Church T. (2015). Light Intensity physical activity and sedentary behavior in relation to body mass index and grip strength in older adults: cross-sectional findings from the Lifestyle Interventions and Independence for Elders (LIFE) study. PloS one. 10(2): e0116058.

Bao W, Rong Y, Rong S, Liu L: Dietary iron intake, body iron stores, and the risk of type 2 diabetes: a systematic review and meta-analysis. BMC Medicine. 10: 119.

Barbieri M, Ragno E, Benvenuti E. (2001). New aspects of the insulin resistance syndrome: impact on haematological parameters. Diabetologia. 44(10): 1232-1237.

Barker D, Hales C, Fall C, Osmond C, Phipps K, Clark P. (1993). Type 2 (non-insulin-dependent) diabetes mellitus, hypertension and hyperlipidaemia (syndrome X): relation to reduced fetal growth. Diabetologia. 36:62–67.

Barr S, Di Francesco L, Fulgoni V. (2016). Association of breakfast consumption with body mass index and prevalence of overweight/obesity in a nationally-representative survey of Canadian adults. Nutrition journal. 15(1): 33.

Bastard J, Maachi M, Lagathu C, Kim M, Caron M, Vidal H, Capeau J, Feve B. (2006). Recent advances in the relationship between obesity, inflammation, and insulin resistance. European Cytokine Network. 17: 4–12.

Basu A, Du M, Sanchez K, Leyva M, Betts N, Blevins S, Wu M, Aston C, Lyons T. (2011). Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. Nutrition. *2*: 206–213.

Beauchamp B, Ghosh S, Dysart M, Kanaan G, Chu A, Blais A, Rajamanickam K, Tsai E, Patti M, Harper M. (2015). Low birth weight is associated with adiposity, impaired skeletal muscle energetics, and weight loss resistance in mice. International journal of obesity. 39(4): 702.

Berg A, Scherer P. (2005). Adipose tissue, inflammation, and cardiovascular disease. Circulation Research. 96(9): 939–949.

Berg C, Lappas G, Wolk A, Strandhagen E, Toren K, Rosengren A, et al. (2009). Eating patterns and portion size associated with obesity in a Swedish population. Appetite. 52: 21–26.

Bhurosy T, Jeewon R. (2014). Overweight and obesity epidemic in developing countries: A problem with diet, physical activity, or socioeconomic status? The Scientific World Journal. Volume 2014, Article ID 964236, 7 pages http://dx.doi.org/10.1155/2014/964236

Bigaard J, Frederiksen K, Tjønneland A, Thomsen B, Overvad K, Heitmann B *et al*. (2005). Waist circumference and body composition in relation to all-cause mortality in middle-aged men and women. International Journal of Obesity (London). 29: 778–784

Bleich S, Wang Y, Wang Y, Gortmaker S. (2009). Increasing consumption of sugar-sweetened beverages among us adults: 1988–1994 to 1999–2004. American Journal of Clinical Nutrition. 89: 372–381.

Bogdanski P, Suliburska J, Szulinska M, Stepien M, Pupek-Musialik D, Jablecka A. (2012). Green tea extract reduces blood pressure, inflammatory biomarkers, and oxidative stress and improves parameters associated with insulin resistance in obese, hypertensive patients. Nutrition Research. 6: 421–427.

Booth A, Magnuson A, Fouts J, Foster M. (2016). Adipose tissue: an endocrine organ playing a role in metabolic regulation. Hormone molecular biology and clinical investigation. 26(1): 25-42.

Bouchard D, Ross R, Janssen I. (2010). Coffee, Tea and their additives: association with BMI and waist circumference. Obesity Facts. 3: 345-352

Bray GA, Popkin BM. (2014). Dietary sugar and body weight: have we reached a crisis in the epidemic of obesity and diabetes?: health be damned! Pour on the sugar. Diabetes Care. 37: 950–956

Brooks G, Blaha M, Blumenthal R. (2010). Relation of C-reactive protein to abdominal adiposity. American Journal of Cardiology. 106: 56–61.

Brown A, Bohan Brown M, Allison D. (2013). Belief beyond the evidence: using the proposed effect of breakfast on obesity to show 2 practices that distort scientific evidence. American Journal of Clinical Nutrition. 98:1298–1308.

Bulló M, García-Lorda P, Megias I, Salas-Salvadó J. (2003) Systemic inflammation, adipose tissue tumor necrosis factor, and leptin expression. Obesity Research. 11: 525–531.

Buyukkaya E, Karakas M, Karakas E, Akcay A, Tanboga I, Kurt M, Sen N. (2012). Correlation of neutrophil to lymphocyte ratio with the presence and severity of metabolic syndrome. Clinical and Applied Thrombosis/Hemostasis. 20(2): 159 – 163

Caballero A. (2003). Endothelial dysfunction in obesity and insulin resistance: a road to diabetes and heart disease. Obesity Research. 11(11): 1278–1289.

Carney M. (1988). Diethylpropion and psychosis. Clinical Neuropharmacology. 11: 183 – 188.

Carrera-Bastos P, Fontes-Villalba M, O'Keefe J, Lindeberg S, Cordain L. (2011). The western diet and lifestyle and diseases of civilization. Research Reports in Clinical Cardiology. 2: 15-35.

Casabiell X, Piñeiro V, Tomé M, Peinó R, Dieguez C, Casanueva F. (1997). Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. The Journal of Clinical Endocrinology & Metabolism. 82(12): 4270-4273.

Casazza K, Fontaine F, Astrup A, Birch L, Brown A, Bohan Brown M, et al. (2013). Myths, presumptions, and facts about obesity. New England Journal of Medicine. 368: 446–454.

Caveney E, Caveney B, Somaratne R, Turner J, Gourgiotis L, (2011). Pharmaceutical interventions for obesity: a public health perspective. Diabetes, Obesity and Metabolism. 13(6): 490-497.

Cawley J, Meyerhoefer C, Biener A, Hammer M, Wintfeld N. (2014). Savings in medical expenditures associated with reductions in body mass index among US adults with obesity, by diabetes status. Pharmacoeconomics. 33(7): 707 - 722

Celi F, Bini V, De Giorgi G, Molinari D, Faraoni F, Di Stefano G, Bacosi M, Berioli M, Contessa G, Falorni A. (2003). Epidemiology of overweight and obesity among school children and adolescents in three provinces of central Italy, 1993–2001: study of potential influencing variables. The European Journal Clinical Nutrition. 57: 1045–1051

Cha K, Song D, Kim S, Pan C. (2012). Inhibitors of gastronintestinal lipoluss by geen tea, coffee and gomchui (Ligularia fischeri) tea polyphenols during simulated digestion. Journal of Agricultural and food chemistry. 60: 7152- 7157

Chan D, Watts G, Barrett P, Mamo J, Redgrave T. (2002). Markers of triglyceride-rich lipoprotein remnant metabolism in visceral obesity. Clinical Chemistry. 48: 278–283.

Chaput J, Klingenberg L, Sjödin A. (2011). Modern Sedentary Activities Promote Overconsumption of Food in our Current Obesogenic Environment. Obesity Reviews. 12(5), p12-20

Chen Y, Chen P, Hsieh W, Portnov B, Chen Y, Lee Y. (2012). Environmental factors associated with overweight and obesity in Taiwanese children. Paediatric Prenatal Epidemiology. 26: 561–571

Chen Y, Cheung C, Reuhl K, Liu A, Lee M, Lu Y, Yang C. (2011). Effects of green tea polyphenol (-)-epigallocatechin-3-gallate on newly developed high-fat/Western-style diet-induced obesity and metabolic syndrome in mice. Journal of Agriculture and Food Chemistry. 21: 11862–11871.

Chiva-Blanch G, Badimon L, Estruch R. (2014). Latest evidence of the effects of the Mediterranean diet in prevention of cardiovascular disease. Current Atherosclerosis Reports. 16: 446

Cho GJ, Yoo SW, Hong SC. (2006). Correlations between umbilical and maternal serum resistin levels and neonatal birth weight. Acta Obstetricia et Gynecologica Scandinavica. 85(9): 1051-1056.

Chou YC, Ho CT, Pan MH. (2018). Stilbenes: Chemistry and Molecular Mechanisms of Anti-obesity. Current Pharmacology Reports. 1-8.

Coban E, Ozdogan M, Yazicioglu G, Akcit F. (2005). The mean platelet volume in patients with obesity. International Journal of Clinical Practice. 59: 981–982.

Colquitt J, Pickett K, Loveman E, Frampton G. (2014). Surgery for weight loss in adults. Cochrane Database System Review.

Colquitt J, Picot J, Loveman E, Clegg A. (2009). Surgery for obesity. Cochrane Database Systematic reviews.

Cook J, Lipschitz D, Miles L, Finch C. (1974). Serum ferritin as a measure of iron stores in normal subjects. American Journal of Clinical Nutrition. 27: 681–687.

Dalmas E, Venteclef N, Caer C, Poitou C, Cremer I, Aron-Wisnewsky J, Lacroix-Desmazes S, et al. (2014). T Cell–derived IL-22 amplifies IL-1β–driven inflammation in human adipose tissue: Relevance to obesity and type 2 diabetes. Diabetes. 63(6): 1966-1977.

De Boer AA, Monk JM, Liddle DM, Hutchinson AL, Power KA, Ma DW, Robinson LE. (2016). Fish-oil-derived n-3 polyunsaturated fatty acids reduce NLRP3 inflammasome activity and obesity-related inflammatory cross-talk between adipocytes and CD11b+ macrophages. The Journal of nutritional biochemistry. 34: 61-72.

de la Garza A, Milagaro F, Boque N, Campion J, Martinez J. (2011). Natural inhibitors of pancreatic lipase as new players in obesity treatment. Planta medica. 77: 773- 785

Dehghani M, Rezaei Y, Fakour S, Arjmand N. (2016). White blood cell count to mean platelet volume ratio is a prognostic factor in patients with non-ST elevation acute coronary syndrome with or without metabolic syndrome. Korean Circulation Journal. 46(2): 229-238.

Del Giudice M, Leonardi S, Ciprandi G, Galdo F, Gubitosi A, La Rosa M. (2012). Probiotics in childhood: allergic illness and respiratory infections. Journal of Clinical Gastroenterology. 46: 69–72.

Deshmukh-Taskar P, Nicklas T, Radcliffe J, O'Neil C, Liu Y. (2013). The relationship of breakfast skipping and type of breakfast consumed with overweight/obesity, abdominal obesity, other cardiometabolic risk factors and the metabolic syndrome in young adults: The National Health and Nutrition Examination Survey (NHANES): 1999–2006. Public Health Nutrition. 11:2073–2082.

Després J, Moorjani S, Lupien P, Tremblay A , Nadeau A , Bouchard C. (1990). Regional distribution of body fat, plasma lipoproteins, and cardiovascular disease. Arteriosclerosis. 10: 497–511.

Dewey K, Lonnerdal B. (1986). Infant self-regulation of breast milk intake. Acta Paediatrica Scandinavica. 75: 893-898.

Dewey K, Nommsen-Rivers L, Heinig M, Cohen R. (2003). Risk factors for suboptimal infant breastfeeding behaviour, delayed onset of lactation, and excess neonatal weight loss. Paediatrics. 112(3): 607-619.

Dewey K. (1998). Growth characteristics of breast-fed compared to formula fed infants. Biology of Neonate. 74: 94–105.

Dietz W. (2001). Breastfeeding may help prevent childhood overweight. Journal of the American Medical Association. 285: 2506–2507.

Dolan M, Sorkin J, Hoffman D. (2007). Birth weight is inversely associated with central adipose

tissue in healthy children and adolescents. Obesity (Silver Spring). 15: 1600–1608.

Donini L, Cuzzolaro M, Spera G, Badiali M , Basso N , Bollea M, Bosello O , Brunani A , Busetto L , Cairella G , Cannella C , Capodaglio P , Carbonelli M , Castellaneta E , Castra R , Clini E , Contaldo F , Dalla Ragione L , Dalle Grave R , D'Andrea F , Del Balzo V , De Cristofaro P , Di Flaviano E , Fassino S , Ferro AM , Forestieri P , Franzoni E , Gentile M , Giustini A , Jacoangeli F , Lubrano C , Lucchin L , Manara F , Marangi G , Marcelli M , Marchesini G , Marri G , Marrocco W , Melchionda N , Mezzani B , Migliaccio P , Muratori F , Nizzoli U , Ostuzzi R , Panzolato G , Pasanisi F , Persichetti P , Petroni ML , Pontieri V , Prosperi E , Renna C , Rovera G , Santini F , Saraceni V , Savina C , Scuderi N , Silecchia G , Strollo F , Todisco P , Tubili C , Ugolini G , Zamboni M. (2010). Obesity and eating disorders. Indications for the different levels of care. An Italian expert consensus document. Eating and Weight Disorders. 15(1-2). 1–31.

Dorotsy A, Emmett P, Cowin S, Reilly J, Team A. (2000). Factors associated with early adiposity rebound. Paediatrics. 105: 1115-1118.

Dulloo A, Duret C, Rohrer D, Girardier L, Mensi N, Fathi M, Chantre P, Vandermander J. (1999). Efficacy of a green tea extract rich in catechin polyphenols and caffeine in increasing 24-h energy expenditure and fat oxidation in humans. The American Journal of Clinical Nutrition. 70(6): 1040-1045

Eckel RH, Grundy SM, Zimmet PZ. (2005). The metabolic syndrome. The lancet. 365(9468): 1415-1428.

Egger G, Cameron-Smith D, Stanton R. (1999). The effectiveness of popular, non-prescription weight loss supplements. Medical Journal of Australia. 171: 604–608.

Eknoyan G. (2008). Adolphe Quetelet (1796–1874)—the average man and indices of obesity. Nephrology Dialysis Transplantation 23(1): 47-51.

Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. (2014). Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. Diabetes Research and Clinical Practice. 105: 141–50.

Fabry P, Hejl Z, Fodor J, Braun T, Zvolankova K. (1964). The frequency of meals: its relation to overweight, hypercholesterolaemia, and decreased glucosetolerance. Lancet. 2: 614–615.

Fadini G, Marcuzzo G, Marescotti M. (2012). Elevated white blood cell count is associated with prevalence and development of the metabolic syndrome and its components in the general population. Acta Diabetologica. 49(6): 445-451.

Fain J, Madan A, Hiler, M, Cheema P, Bahouth S. (2004). Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology. 145(5): 2273-2282.

Feijo M, Ballard CR, Foletto KC, Batista BA, Neves AM, Ribeiro MF, et al. (2013). Saccharin and aspartame, compared with sucrose, induce greater weight gain in adult Wistar rats, at similar total caloric intake levels. Appetite. 60(1): 203–207.

Fernandez-Riejos P, Najib S, Santos-Alvarez J, Martin-Romero C, Perez-Perez A, Gonzalez-Yanes C, SanchezMargalet V. (2010). Role of leptin in the activation of immune cells. Mediators of Inflammation. 2010: 1-8.

Ferrante A. (2007). Obesity-induced inflammation: a metabolic dialogue in the language of inflammation. Journal of Internal Medicine. 262: 408–414.

Festa A, D'Agostino R, Williams K et al. (2001). The relation of body fat mass and distribution to markers of chronic inflammation. International Journal of Obesity Related Metabolic Disorders 25: 1407–1415.

Finkelstein E, Trogdon J, Cohen J, Dietz W. (2009) Annual medical spending attributable to obesity: payer- and service-specific estimates. Health Affairs. 28(5):822 -831.

Finkelstein Eric, Olga K, Thompson H, Trogdon J, Pan L, Sherry B, Dietz W. (2012). Obesity and severe obesity forecasts through 2030. American journal of preventive medicine. 42(6): 563-570.

Fisch I, Freedman S. (1975). Smoking, oral contraceptives, and obesity. Effects on white blood cell count. JAMA. 234: 500-506

Frasca D, Diaz A, Romero M, Vazquez T, Blomberg BB. (2017). Obesity induces pro-inflammatory B cells and impairs B cell function in old mice. Mechanisms of ageing and development. 162: 91-99.

French S, Story M, Jeffery R. (2001). Environmental influences on eating and physical activity. Annual Review of Public Health. 22: 309–335.

Friedman J, Halaas J. (1998). Leptin and the regulation of body weight in mammals. Nature.

395(6704): 763-770.

Friedrich M, Petzke K, Raederstorff D, Wolfram S, Klaus S. (2012). Acute effects of epigallocatechin gallate from green tea on oxidation and tissue incorporation of dietary lipids in mice fed a high-fat diet. International Journal of. Obesity. 36: 735–743

Frye C, Heinrich J. (2003). Trends and predictors of overweight and obesity in East German children. The International Journal of Obesity and Related Metabolic Disorders. 27: 963–969.

Gandhi G, Jothi G, Antony P, *et al*. (2014). Gallic acid attenuates high-fat diet fed streotozotochin-induced insulin resistance via partial agonism of PPAR$_{\gamma}$ in experimental type 2 diabetic rats and enhances glucose uptake through translocation and activation of GLUT4 In PI3K/*p*-Akt signalling pathway. European Journal of Pharmacology. 745: 201-216

García-Lafuente A, Guillamón E, Villares A, Rostagno M, Martínez J. (2009). Flavonoids as anti-inflammatory agents: implications in cancer and cardiovascular disease. Inflammation Research. 58: 537-552

García-Villalba R, Carrasco-Pancorbo A, Nevedomskaya E, Mayboroda O, Deelder, Segura-Carretero A, Fernansez-Gutierrez A. (2010). Exploratory analysis of human urine by LC-ESI-TOF MS after high intake of olive oil: understanding the metabolism of polyphenols. Analytical and Bioanalyitcal Chemistry. 398: 463–75.

Gerhardt C, Romero I, Cancello R, Camoin L, Strosberg A. (2001). Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. Molecular and Cellular Endocrinology. 175: 81–92.

Gillum R. (2001). Association of serum ferritin and indices of body fat distribution and obesity in Mexican American men–the Third National Health and Nutrition Examination Survey. International Journal of Obesity Related Metabolic Disorder. 25: 639–645.

Gluckman PD, Hanson MA, Spencer HG. (2005). Predictive adaptive responses and human evolution. Trends in Ecology and Evolution. 20(10): 527–533.

Graham J, Ryall R, Wise P. (1980). Glycosylated haemoglobin and relative polycythaemia in diabetes mellitus. Diabetologia. 18(3): 205-207.

Grant R, Dixit V. (2015). Adipose tissue as an immunological organ. Obesity. 23(3): 512-518.

Greenberg J, Axen K, Schnoll R, Boozer C. (2005). Coffee, tea and diabetes: the role of weight loss and caffeine. International Journal of Obesity (London). 29: 1121–1129.

Gregor M, Hotamisligil G. (2011). Inflammatory Mechanisms in Obesity. Annual Reviews. 29: 415-445.

Grujic V, Cvejin M, Nikolic E, Dragnic N, Jovanovic V, Kvrgic S, et al. (2009). Association between obesity and socioeconomic factors and lifestyle. Vojnosanitetski Pregled. 66: 705–710.

Guo Y, Wu G, Su X, Yang H, Zhang J. (2009). Antiobesity action of daidzein derivative on male obese mice induced by a high-fat diet. Nutrition research (N.Y.). 29: 656- 663.

Hales C, Barker D. (1992). Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. Diabetologia. 35: 595-601.

Halton T, Willett W, Liu S, Manson J, Stampfer M, Hu F. (2006). Potato and french fry consumption and risk of type 2 diabetes in women. The American Journal of Clinical Nutrition. 83(2): 284-290.

Han L, Kimura Y, Kawashima M, Takaku T, Taniyama T, Hayashi T, Zheng Y, Okuda H. (2001). Anti-obesity effects in rodents of dietary tea saponin, a lipase inhibitor. International Journal of Obesity Related Metabolic Disorders. 25: 1459-1464.

Han T, Lean M, (2016). A clinical perspective of obesity, metabolic syndrome and cardiovascular disease. Journal of the Royal Society of Medicine, Cardiovascular Disease. 5: 1-13.

Heinig M, Nommsen L, Peerson J, Lonnerdal B, Dewey K. (1993). Energy and protein intakes of breast-fed and formula-fed infants during the first year of life and their association with growth velocity: the DARLING study. The American Journal of Clinical Nutrition. 58: 152-161.

Heinonen I, Rinne P, Ruohonen ST, Ruohonen S, Ahotupa M, Savantaus E. (2014). The effect of equal caloric high fat and western diet on metabolic syndrome, oxidative stress and vascular endothelial function in mice. Acta Physiologica. 211: 515-527

Higdon J, Frei B. (2006). Coffee and health: a review of recent human research. Critical Review of Food Science and Nutrition. 46: 101–123.

Hill J, Commerford R. (1996). Physical activity, fat balance, and energy balance. International Journal of Sport Nutrition. 6(2): 80–92.

Hjelmborg J, Fagnani C, Silventoinen K, McGue M, Korkeila M, Christensen K, Rissanen A, Kaprio J. (2008) Genetic influences on growth traits of BMI: a longitudinal study of adult twins. Obesity. 16: 847–852.

Ho S, Lam T, Janus E. (2003). The Hong Kong Cardiovascular Risk Factor Prevalence Study steering committee. Waist to stature ratio is more strongly associated with cardiovascular risk factors than other simple anthropometric indices. Annals of Epidemiology. 13: 683–691.

Horta B, Bahl R, Martines J, Victora C. (2007). Evidence on the long-term effects of breastfeeding. Systematic reviews and meta-analysis. Geneva: World Health Organization. ISBN 978-92-4-159523-0.

Hotamisligil G. (2006). Inflammation and Metabolic Disorders. Nature. 444: 860–867.

Houseknecht K, McGuire M, Portocarrero C, McGuire M, Beerman K. (1997). Leptin is present in human milk and is related to maternal plasma leptin concentration and adiposity. Biochemical and Biophysical Research Communications. 240: 742–747.

Hsieh C, Pei D, Kuo S, Chen C, Tsai S, Lai C, Lee C, Wu C, Hung Y, Hsieh M. (2007). Correlation between white blood cell count and metabolic syndrome in adolescence. Paediatrics International. 49(6): 827-832.

Hsieh S, Yoshinaga H. (1995). There any difference in coronary heart disease risk factors and prevalence of fatty liver in subjects with normal body mass index having different physiques? The Tohoku journal of experimental medicine. 177.3: 223-231.

Hu F. (2007). Obesity and mortality: watch your waist, not just your weight. Archive Internal Medicine. 167(9): 875-876.

Huang C-J, Hu H-T, Fan Y-C, Liao Y-M, Tsai P. (2010). Associations of breakfast skipping with obesity and health-related quality of life: evidence from a national survey in Taiwan. International Journal of Obesity. 34: 720–725.

Huang S, Rutkowsky JM, Snodgrass RG, Ono-Moore KD, Schneider DA, Newman JW, Adams SH, Hwang DH. (2012). Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. The Journal of Lipid Research. 53: 2002–2013.

Ilavska S, Horvathova M, Szabova M. (2012). Association between the human immune response and body mass index. Human Immunology. 73(5): 480-485.

Ilcol Y, Hizli Z, Eroz E. (2008). Resistin is present in human breast milk and it correlates with maternal hormonal status and serum level of C-reactive protein. Clinical Chemistry and Laboratory Medicine. 46(1): 118-124.

Ioannides-Demos L, Proietto J, Tonkin A, McNeil J. (2006). Safety of drug therapies used for weight loss and treatment of obesity. Drug Safety. 29: 277–302.

Ip S, Chung M, Raman G. (2007). Tufts-New England Medical Center Evidence-based Practice Center. Breastfeeding and maternal and infant health outcomes in developed countries. Evidence report/technology assessment. 153(153): 1–186.

Iwasaki T, Nakajima A, Yoneda M, Yamada Y, Mukasa K, Fujita K, Fujisawa N, Wada K, Terauchi Y. (2005). Serum ferritin is associated with visceral fat area and subcutaneous fat area. Diabetes care. 28(10): 2486-2491.

Iyer A, Fairlie D, Prins J, Hammock B, Brown L. (2010). Inflammatory lipid mediators in adipocyte function and obesity. Nature Reviews Endocrinology. 6: 71– 82.

Jackson K, Nazar A. (2006). Breastfeeding, the immune response, and long-term health. The Journal of the American Osteopathic Association. 106(4): 203-207.

James W, Astrup A, Finer N, Hilsted J, Kopelman P, Rossner S, Saris W, VanGaal L. (2000). Effect of sibutramine on weight maintenance after weight loss: a randomised trial. STORM study group. Sibutramine trial of obesity reductionand maintenance. Lancet. 356: 2119–2125.

Jansses I, Katzmarzyk P, Ross P. (2004). Waist circumference and not body mass index explains obesity-related health risk. American Journal of Clinical Nutrition. 79: 379–384.

Jeffery R, Epstein L, Wilson G, Drewnowski T, Stunkard A, Wing A, Rena R.(2000) Long-term maintenance of weight loss: current status. Health Psychology. 19: 5–16.

Jiao J, Xu JY, Zhang W, Han S, Qin LQ. (2015). Effect of dietary fiber on circulating C-reactive protein in overweight and obese adults: a meta-analysis of randomized controlled trials. International journal of food sciences and nutrition. *66*(1): 114-119.

Jick H. (2000). Heart valve disorders and appetite-suppressant drugs. Journal of the American Medical Association. 283: 1738 – 1740.

Johnson A, Milner J, Makowski L. (2012). The inflammation highway: metabolism accelerates inflammatory traffic in obesity. Immunological Reviews. 249(1): 218-238.

Jones A. (2013). Triglycerides and cardiovascular risk. Heart. 99: 1-2.

Jurado-Ruiz, E, Varela LM, Luque A, Berná G, Cahuana G, Martinez-Force E, Gallego-Durán R, Soria B, Roos B, Romero Gómez M, Martín F. (2017). An extra virgin olive oil rich diet intervention ameliorates the nonalcoholic steatohepatitis induced by a high-fat “Western-type” diet in mice. Molecular nutrition & food research. *61*(3).

Kahn H, Narayan K, Williamson D, Valdez R. (2000). Relation of birth weight to lean and fat thigh tissue in young men. International Journal of Obesity and Related Metabolic Disorder. 24(6): 667-672.

Kalupahana S, Moustaid-Moussa N. (2012). The adipose tissue renin-angiotensin system and metabolic disorders: a review of molecular mechanisms. Critical Review of Biochemistry and Molecular Biology. 47: 379–90.

Karalis K, Giannogonas P, Kodela E, Koutmani Y, Zoumakis M, Teli T. (2009). Mechanisms of obesity and related pathology: linking immune responses to metabolic stress. FEBS Journal. 276: 5747–5754.

Karlsson EA, Hertz T, Johnson C, Mehle A, Krammer F, Schultz-Cherry S. (2016). Obesity outweighs protection conferred by adjuvanted influenza vaccination. MBio. *7*(4): e01144-16.

Karu N, Reinfen R, Kerem Z. (2007). Weight gain reduction in mice fed Panax ginseng saponin, a pancreatic lipase inhibitor. Journal of agricultural and food chemistry. 55: 2824- 2828.

Kassi E, Pervanidou P, Kaltsas G, Chrousos G. (2011). Metabolic syndrome: definitions and controversies. BMC Medicine. 9: 48.

Katsuki A, Sumida Y, Urakawa H, Gabazza E, Murashima S, Maruyama N, Morioka K, Nakatani K, Yano Y, Adachi Y. (2003). Increased visceral fat and serum levels of triglyceride are associated with insulin resistance in Japanese metabolically obese, normal weight subjects with normal glucose tolerance. Diabetes Care. 26: 2341–2344.

Klimentidis Y, Beasley M, Lin H, Murati G, Glass G, Guyton M, Newton W, Jorgensen M, Heymsfield S, Kemnitz J, Fairbanks L, Allison D. (2011). Canaries in the coal mine: a cross-species analysis of the plurality of obesity epidemics. Proceedings of the Royal Society B. 278(1712): 1626–1632.

Kovacs E, Lejeune M, Nijs I, Westerterp-Plantenga M. (2004). Effects of green tea on weight maintenance after body-weight loss. British Journal of Nutrition. 91(3): 431-437.

Kuksis A, Myher J, Geher K, Breckenridge W, Feather T, McGuire V, Little J. (1991). Gas chromatographic profiles of plasma total lipids as indicators of dietary history. Correlation with Fat Intake Based on 24-H Dietary Recall. Journal of Chromatography. 564: 11-26.

Kullo I, Hensrud D, Allison T. (2002). Comparison of numbers of circulating blood monocytes in men grouped by body mass index (<25, 25 to <30, > or = 30). American Journal of Cardiology. 89: 1441–1443.

Kulovitz M, Kravitz L, Mermier C, Gibson A, Conn C, Kolkmeyer D, Kerksick C. (2014). Potential role of meal frequency as a strategy for weight loss and health in overweight or obese adults. Nutrition. 30(4): 386-392.

Kunutsor S, Apekey T, Walley J, Kain K. (2013). Ferritin levels and risk of type 2 diabetes mellitus: an updated systematic review and meta-analysis of prospective evidence. Diabetes Metabolism Research Review. 29: 308–318.

Kushner R. (2014). Weight loss strategies for treatment of obesity. Progress in cardiovascular diseases. 56(4): 465-472.

Kwok M, Schooling C, Lam T. (2010). Does breastfeeding protect against childhood overweight? Hong Kong's 'Children of 1997' birth cohort. International Journal of Epidemiology. 39: 297–305.

Kyle J, Duthie G, Andersen O, Markhamv K. (2006). Flavonoids in Foods in Flavonoids. Chemistry, Biochemistry and Applications. CRC Press, Boca Raton. pp: 219.

Labayen I, Moreno L, Ruiz J, González-Gross M, Wärnberg J, Breidenassel C, Ortega F, Marcos A, Bueno M. (2008). Small birth weight and later body composition and fat distribution in adolescents: the Avena study. Obesity Society. 16(7): 1680-1686.

Larsen CM, Faulenbach M, Vaag A, et al. (2007). Interleukin-1-receptor antagonist in type 2 diabetes mellitus. New England Journal of Medicine. 356: 1517–1526

Latha RC, Daisy P. (2011). Insulin-secretagogue, antihyperlipidemic and other protective effets of gallic acid isolated from Terminalia bellerica Roxb. In streptozotocin-induced diabetic rats. Chemico-Biological Interactions. 189: 112-118

Law C, Barker D, Osmond C, Fall C, Simmonds S. (1992). Early growth and abdominal fatness in

adult life. Journal of Epidemiology, Community Health. 46: 184–186.

Lee J, Mishra G, Hayashi K, Watanabe E, Mori K, Kawakubo K. (2016). Combined eating behaviors and overweight: Eating quickly, late evening meals, and skipping breakfast. Eating behaviours. 2: 84-88.

Lee Y, Cha B, Yamaguchi K, Choi S, Yonezawa T, Teruya T, Nagai K, Woo J. (2010). Effects of Korean white ginseng extracts on obesity in high-fat diet-induced obese mice. Cytotechnology. 62: 367-376.

Lin J, Lin-Shiau S. (2006). Mechanisms of hypolipidemic and anti-obesity effects of tea and tea polyphenols. Molecular nutrition & food research. 50(2): 211-217.

Lopez-Garcia E, van Dam RM, Rajpathak S, Willett W, Manson J, Hu F. (2006). Changes in caffeine intake and long-term weight change in men and women. American Journal of Clinical Nutrition. 83: 674–680.

Lowe M. (1994). Pancreatic triglyceride lipase and colipase: insights into dietary fat digestion. Gastroenterology. 107: 1524- 1536.

Lu C, Zhu W, Shen C, Gao W. (2012). Green tea polyphenols reduce body weight in rats by modulating obesity-related genes. PLoS One. 7(6): 1-11.

Lubrano C, Saponara M, Barbaro G, Specchia P, Addessi E, Costantini D, Tenuta M, Di Lorenzo G, Genovesi G, Donini L, Lenzi A, Gnessi L. (2012). Relationships between body fat distribution, epicardial fat and obstructive sleep apnea in obese patients with and without metabolic syndrome. PLoS ONE7(10): e47059. https://doi.org/10.1371/journal.pone.0047059

Lucas A, Boyes S, Bloom R, Aynsley-Green A. (1981). Metabolic and endocrine responses to a milk feed in six-day old term infants: differences between breast and cow's milk formula feeding. Acta Paediatrica Scandinavica. 70: 195-200.

Ma Y, Bertone E, Stanek E 3rd, Reed G, Hebert J, Cohen N, et al. Association between eating patterns and obesity in a free-living US adult population. American Journal of Epidemiology. 15: 85–92.

Maki K, Reeves M, Farmer M, Yasunaga K, Matsuo N, Katsuragi Y, Komikado M, Tokimitsu I, Wilder D, Jones F, Blumberg J, Cartwright Y. (2009). Green tea catechin consumption enhances

exercise-induced abdominal fat loss in overweight and obese adults. Journal of Nutrition. 139: 264–270.

Makki K, Froguel P, Wolowczuk I. (2013). Adipose tissue in obesity-related inflammation and insulin resistance: cells, cytokines, and chemokines. *ISRN inflammation*. *2013*: http://dx.doi.org/10.1155/2013/139239

Mardi T, Toker S, Melamed S. (2005). Increased erythropoiesis and subclinical inflammation as part of the metabolic syndrome. Diabetes Research and Clinical Practice. 69(3): 249-255.

Marseglia L, Manti S, D'Angelo G, Cuppari C, Salpietro V, Filippelli M, Trovato A, Gitto E, Salpietro C, Arrigo T. (2015). Obesity and breastfeeding: the strength of association. Women and Birth. 28(2): 81-86.

Martí A, Marcos A, Martínez J. (2001). Obesity and immune function relationships. Obesity Reviews. 2: 131–140.

Martin L, Woo J, Geraghty S. (2006). Adiponectin is present in human milk and is associated with maternal factors. The American Journal of Clinical Nutrition. 83(5): 1106-1111.

Martin LJ, Woo JG, Geraghty SR. (2006). Adiponectin is present in human milk and is associated with maternal factors. The American Journal of Clinical Nutrition. 83 (5): 1106-1111.

Martínez-González M, Salas-Salvadó J, Estruch R, Corella D, Fito M, Ros E. (2015). Benefits of the Mediterranean diet: insights from the PREDIMED study. Progressive Cardiovascular Disease. 58: 50–60.

McCance R. (1962). Food, growth and time. Lancet. 2: 671–676.

McDougall G, Kulkarni N, Stewart D. (2008). Current developments on inhibitory effects of berry polyphenols on digestive enzymes. Biofactors. 34: 73- 80.

Means R. (2004). Hepcidin and anaemia. Blood Reviews. 18(4): 219-225.

Medzhitov R. (2008). Origin and physiological roles of inflammation. *Nature*. 454(7203): 428–435.

Miralles O, Sanchez J, Palou A, Pico C. (2006). A physiological role of breast milk leptin in body weight control in developing infants. Obesity (Silver Spring). 14: 1371–1377.

Mittendorfer B, Yoshino M, Patterson BW, Klein S. (2016). VLDL triglyceride kinetics in lean, overweight, and obese men and women. The Journal of Clinical Endocrinology & Metabolism. 101(11): 4151-4160.

Miyagawa S, Kobayashi M, Konishi N. (2000). Insulin and insulin-like growth factor I support the proliferation of erythroid progenitor cells in bone marrow through the sharing of receptors. British Journal of Haematology. 109(3): 555-562.

Mohammadreza V, Nazanin M, Shirin A, Aghafatemeh H, Mohammadreza E. (2012). Relationship between breastfeeding and obesity in childhood. Journal of Health, Population and Nutrition. 30: 303–310.

Monasta L, Batty G, Cattaneo A, Lutje V, Ronfani L, Van Lenthe F, Brug J. (2010). Early-life determinants of overweight and obesity: a review of systematic reviews. Obesity Review. 11: 695–708.

Moore LL, Singer MR, Qureshi MM, Bradlee ML. (2008). Dairy intake and anthropometric measures of body fat among children and adolescents in NHANES. Journal of the American College of Nutrition. *27*(6): 702-710.

Motilva M, Serra A, Macià A. (2013). Analysis of food polyphenols by ultra high-performance liquid chromatography coupled to mass spectrometry: an overview. Journal of Chromatography A. 1296: 66-82.

Mozaffarian D, Hao T, Rimm EB, Willett WC, Hu FB. (2011). Changes in diet and lifestyle and long-term weight gain in women and men. New England Journal of Medicine. 364: 2392–2404.

Nagao T, Komine Y, Soga S, Meguro S, Hase T, Tanaka Y, Tokimitsu I. (2005). Ingestion of a tea rich in catechins leads to a reduction in body fat and malondialdehyde-modified LDL in men. American Journal of Clinical Nutrition. 81: 122–129.

Nagasawa N, Tamakoshi K, Yatsuya H et al. (2004). Association of white blood cell count and clustered components of metabolic syndrome in Japanese men. Circculation Journal. 68: 892-897.

Naksi M, Fukui Y, Asami S, Toyoda-Ono Y, Iwashita T, Shibata H, Mitsunaga T, Hashimoto F, Kiso Y. (2005). Inhibitory effects of oolong tea polyphenols and pancreatic lipase in vitro. Journal of agricultural and food chemistry. 53: 4593- 4598.

National Institute for Health Care Excellence. (2014). https://www.nice.org.uk/guidance/cg189 Available: Obesity: identification, assessment and management. Last accessed 09/07/2017.

National Institutes of Health, National Heart, Lung, and Blood Institute. (1998). Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: the evidence report. Obesity Research. 6: 51–209.

NHS. (2016). What is the body mass index (BMI)? Available: http://www.nhs.uk/chq/Pages/3215.aspx?CategoryID=52 . Last accessed 09/07/2017.

Nicklas J, Huskey K, Davis R, Wee C. (2012). Successful weight loss among obese US adults. American Journal of Preventative Medicine. 42: 481-485.

Nishimura S, Manabe I, Nagasaki M, Seo K, Yamashita H, Hosoya Y, Ohsugi M, Tobe K, Kadowaki T, Nagai R, Sugiura S. (2014). In vivo imaging in mice reveals local cell dynamics and inflammation in obese adipose tissue. Journal of Clinical Investigation. 118: 710–721.

O'Neil C, Nicklas T, Fulgoni III V. (2014). Nutrient intake, diet quality, and weight/ adiposity parameters in breakfast patterns compared with no breakfast in adults: National Health and Nutrition Examination Survey 2001–2008. Journal of the Academy of Nutrition and Dietetics. 114(3): 27–43.

Odagiri K, Uehara A, Mizuta I, Yamamoto M, Kurata C. (2011). Longitudinal study on white blood cell count and the incidence of metabolic syndrome. Internal Medicine. 50: 2491–2498. doi:10.2169/internalmedicine.50.5877

Odeleye O, de Courten M, Pettitt D, Ravussin E. (1997). Fasting hyperinsulinemia is a predictor of increased body weight gain and obesity in Pima Indian children. Diabetes. 46: 1341-1345.

Ogden C, Yanovski S, Carroll M, Flegal K. (2007). The epidemiology of obesity. Gastroenterology. 132: 2087–2102.

Ohkawara K, Cornier M, Kohrt W, Melanson E. (2013). Effects of increased meal frequency on fat oxidation and perceived hunger. Obesity. 21(2): 336-343.

Ouchi N, Parker J, Lugus J, Walsh K. (2011). Adipokines in inflammation and metabolic disease. Nature Reviews Immunology. 11(2): 85–97.

Owen C, Martin R, Whincup P, Smith G, Cook D. (2005). Effect of infant feeding on the risk of obesity across the life course: A quantitative review of published evidence. Paediatrics. 115: 1367–1377.

Owen C, Martin R, Whincup P, Smith G, Cook D. (2006) Does breastfeeding influence risk of type 2 diabetes in later life? A quantitative analysis of published evidence. The American Journal of Clinical Nutrition. 84, 1043–1054.

Padwal R, Li S, Lau D. (2004). Long-term pharmacotherapy for obesity and over-weight. Cochrane Database System Review. 3: 1-99.

Pal D, Dasgupta S, Kundu R, Maitra S, Das G, Mukhopadhyay S, Ray S, Majumdar SS, Bhattacharya S. (2012). Fetuin-A acts as an endogenous ligand of TLR4 to promote lipid-induced insulin resistance. Nature Medicine. 18: 1279–1285.

Palou A, Picó C. (2009). Leptin intake during lactation prevents obesity and affects food intake and food preferences in later life. Appetite. *52*(1): 249-252.

Park H, Park J, Yu R. (2005). Relationship of obesity and visceral adiposity with serum concentrations of CRP, TNF-alpha and IL-6. Diabetes Research and Clinical Practice. 69: 29–35.

Parlee S, MacDougald O. (2014). Maternal nutrition and risk of obesity in offspring: the trojan horse of developmental plasticity. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease. 1842(3): 495-5.

Parsons T, Power C, Manor O. (2001). Foetal and early life growth and body mass index from birth to early adulthood in 1958 British cohort: longitudinal study. British Medical Journal. 323: 1331–1335.

Parsons T, Power C, Manor O. (2003). Infant feeding and obesity through the Life course. Archives of Disease in Childhood. 88: 793–794.

Pasman W, Westerterp-Plantenga M, Saris W. (1997).The effectiveness of long-term supplementation of carbohydrate, chromium, fibre and caffeine on weight maintenance. International Journal of Obesity Related Metabolic Disorders. 21: 1143–1151.

Pérez de Heredia F, Gómez-Martínez S, Marcos A. (2012). Obesity, inflammation and the immune system. Proceedings of the Nutrition Society. 71(2): 332-338.

Pérez-de-Heredia F, Gómez-Martínez S, Díaz L, Veses A, Nova N, Wärnberg J, Huybrechts I, Vyncke K, Androutsos O, Ferrari M, Palacios G, Wastlund A, Kovács E, Gottrand F, González-Gross M, Castillo M, Sjöstrom M, Manios Y, Kafatos A, Molnár D, Widhalm K, Moreno L, Marcos A. (2015). Influence of sex, age, pubertal maturation and body mass index on circulating white blood cell counts in healthy European adolescents—the HELENA study. European Journal of Pediatrics. 174: 999–1014.

Picot J, Jones J, Colquitt J, Gospodarevskaya E, Loveman E, Baxter L, Clegg A. (2009). The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. Health Technology Assess. 13(41):1–190, 215–357, iii-iv. doi: 10.3310/hta13410.

Pi-Sunyer F. (2002). The obesity epidemic: pathophysiology and consequences of obesity. Obesity Research. 10(10): 97–104.

Poobalan A, Aucott L, Smith W, Avenell A, Jung R, Broom J. (2007). Long-term weight loss effects on all cause mortality in overweight/obese populations. Obesity Review. 8: 503-513.

Popkin B. (2006). Global Nutrition dynamics: the world is shifting rapidly towards a diet linked with noncommunicable diseases. The American Journal of Clinical Nutrition. 84 (2), 289-298.

Pories W. (2008). Bariatric surgery: risks and rewards. The Journal of Clinical Endocrinology and Metabolism. 93(11): 89-96.

Poulton R, Williams S. (2001). Breastfeeding and risk of overweight. JAMA. 286: 1449–1450.

Puzziferri N, Roshek TB, Mayo HG, Gallagher R, Belle SH, Livingston EH. (2014). Long-term Follow-up After Bariatric Surgery, A Systematic Review. The Journal of the American medical association. 312(9): 934–942.

Rains T, Agarwal S, Maki K. (2011). Anti-obesity effects of green tea catechins: a mechanistic review. Journal of Nutrition and Biochemistry. 22: 1–7.

Rasmussen F, Johansson M. (1998). The relation of weight, length and ponderal index at birth to body mass index and overweight among 18-year-old males in Sweden. The European Journal of Epidemiology. 14: 373–380.

Ratajczak J, Zhang Q, Pertusini E. (1998). The role of insulin (INS) and insulin-like growth factor-I (IGF-I) in regulating human erythropoiesis. Studies in vitro under serum-free conditions—Comparison to other cytokines and growth factors. Leukemia. 12: 371-381.

Ravelli G, Stein Z, Susser M. (1976). Obesity in young men after famine exposure in utero and early infancy. The New England Journal of Medicine. 295: 349–353.

Reeves S, Halsey L, McMeel Y, Huber J. (2013). Breakfast habits, beliefs and measures of health and wellbeing in a nationally representative UK sample. Appetite. 60:51–57.

Robertson C, Avenell A, Boachie C, Stewart F, Archibald D, Douglas F, Hoddinott P, van Teijlingen E, Boyers D. (2016). Should weight loss and maintenance programmes be designed differently for men? A systematic review of the long-term randomised control trials presenting data for men and women: The ROMEO project. Obesity Research & Clinical Practice. 10(1): 70-84.

Rocha V, Libby P. (2009). Obesity, inflammation, and atherosclerosis. Nature Reviews Cardiology. 6: 399–409.

Rodriguez-Palmero M, Koletzko B, Kunz C, Jensen R. (1999). Nutritional and biochemical properties of human milk: II. Lipids, micronutrients, and bioactive factors. Clinics Perinatology. 26(10): 335–59.

Rogacev K, Ulrich C, Blomer L. (2010). Monocyte heterogeneity in obesity and subclinical atherosclerosis. European Heart Journal. 31(1): 369-376.

Rosen E, Spiegelman B. (2006). Adipocytes as regulators of energy balance and glucose homeostasis. Nature. 444: 847–853.

Rosengren A, Dotevall A, Wilhelmsen L, Thelle D, Johansson S. (2004). Coffee and incidence of diabetes in Swedish women: a prospective 18-year follow-up study. Journal of Internal Medicine. 255: 89–95.

Rugholm S, Baker J, Olsen L, Schack-Nielsen L, Bua J, Sørensen T. (2005). Stability of the association between birth weight and childhood overweight during the development of the obesity epidemic. Obesity Research. 13: 2187–2194.

Sala M, Haller DL, Laferrère B, Homel P, McGinty JJ. (2017). Predictors of Attrition Before and After Bariatric Surgery. Obesity surgery. *27*(2): 548-551.

Salpietro C, Rigoli L, Miraglia Del Giudice M, Cuppari C, Di Bella C, Salpietro A. (2011). TLR2 and

TLR4 gene polymorphisms and atopic dermatitis in Italian children: a multicenter study. International Journal of Immunopathology Pharmacology. 24: 33–40.

Sanchez-Margalet V, Marin-Romero C, Santos-Alvarez J, Goberna R, Najib S, Gonzalez-Yanes C. (2003). Role of leptin as an immunomodulator of blood monocular cells: mechanisms of action. Clinical Experimental Immunology. 133: 11-19.

Schellong K, Schulz S, Harder T, Plagemann A. (2012). Birth weight and long-term overweight risk: systematic review and a meta-analysis including 643,902 persons from 66 studies and 26 countries globally. PloS one. 7(10): 1 -12.

Schmidt F, Weschenfelder J, Sander C, Minkwitz J, Thormann J, Chittka T, Mergl R, Kirkby K, Faßhauer M, Stumvoll M, Holdt L, Teupser D, Hegerl U, Himmerich H. (2015). Inflammatory cytokines in general and central obesity and modulating effects of physical activity. PloS one. 10(3): 1-17.

Schneider C, Segre T: Green tea: potential health benefits. American Family Physician. 79: 591–594.

Silverstone T. (1992). Appetite suppressants. A review. Drugs. 6: 820 – 836.

Simental-Mendía L, Rodríguez-Morán M, Guerrero-Romero F. (2008) The product of fasting glucose and triglycerides as surrogate for identifying insulin resistance in apparently healthy subjects. Metabolic Syndrome Related Disorders. 6: 299–304.

Singhal A, Lanigan J. (2007). Breastfeeding, early growth and later obesity. Obesity reviews. 8(1): 51-54.

Siriwardhana N, Kalupahana N, Cekanova M, Lemieux M, Greer B, Moustaid-Moussa N. (2013). Modulation of adipose tissue inflammation by bioactive food compounds. Journal of Nutrition and Biochemistry. 24:613–623.

Smith-Kirwin S, O'Connor D, De Johnston J, Lancey E, Hassink S, Funanage V. (1998). Leptin expression in human mammary epithelial cells and breast milk. The Journal of Clinical Endocrinology and Metabolism. 83: 1810–1813.

Song W, Chun O, Obayashi A, Cho S, Chung C. (2005). Is consumption of breakfast associated with Body Mass Index in US adults? Journal of the American Dietetic Association. 105: 1373–1382.

Sørensen H, Sabroe S, Rothman K, Gillman M, Fischer P, Sørensen T. (1997). Relation between weight and length at birth and body mass index in young adulthood: cohort study. British Medical Journal. 315: 1137.

Stofkova A. (2009). Leptine and adiponectin: from energy and metabolic dysbalance to inflammation and autoimmunity. Endocrine Regulation. 43: 157–168.

Suliburska J, Bogdanski P, Szulinska M, Stepien M, PupekMusialik D, Jablecka A. (2012). Effects of green tea supplementation on elements, total antioxidants, lipids, and glucose values in the serum of obese patients. Biological Trace Elements Research. 149: 315–322.

te Riele W, Boerma D, Wiezer MJ, Borel Rinkes IH, van Ramshorst B. (2010). Long-term results of laparoscopic adjustable gastric banding in patients lost to follow-up. British Journal of Surgery. 97(10): 1535-1540

Terra X, Montagut G, Bustos M, Llopiz N, Ardvol A, Blad C (2009). Grape-seed procyanidins prevent low-grade inflammation by modulating cytokine expression in rats fed a high-fat diet. Journal of Nutrition and Biochemistry. 20: 210-218.

Thaulow E, Erikssen J, Sandvik L, Stormorken H, Cohn P. (1991). Blood platelet count and function are related to total and cardiovascular death in apparently healthy men. Circulation. 84: 613–617.

Thomas JG, Bond DS, Phelan S, Hill JO, Wing RR. (2014). Weight-loss maintenance for 10 years in the National Weight Control Registry. American journal of preventive medicine. 46(1): 17-23.

Trellakis S, Rydleuskaya A, Fischer C, Canbay A, Tagay S, Scherag A, Bruderek K, Schuler P, Brandau, S. (2012). Low adiponectin, high levels of apoptosis and increased peripheral blood neutrophil activity in healthy obese subjects. Obesity facts. 5(3): 305-318.

Trujillo M, Scherer P. (2006). Adipose tissue-derived factors: impact on health and disease. Endocrine Reviews. 27: 762–778.

Tsuda K, Nishio I. (2004). Leptin and membrane fluidity of erythrocytes in essential hypertension. An electron paramagnetic resonance investigation. American Journal of Hypertension. 17(4): 375-379.

Tsuda K. (2006). Adiponectin and membrane fluidity of erythrocytes in normotensive and hypertensive men. Obesity (Silver Spring). 14(9): 1505-1510.

Tuomilehto J, Hu G, Bidel S, Lindstrom J, Jousilahti P. (2004). Coffee consumption and risk of type 2 diabetes mellitus among middle-aged Finnish men and women. JAMA. 291: 1213–1219.

Tussing-Humphreys L, Pustacioglu C, Nemeth E, Braunschweig C. (2012). Rethinking iron regulation and assessment in iron deficiency, anemia of chronic disease, and obesity: introducing hepcidin. Journal of the Academy Nutrition and Dietetics. 112: 391-400.

UK Department of Health. 2010 to 2015 government policy: obesity and healthy eating. 08/05/2-15. Available at: (https://www.gov.uk/government/publications/2010-to-2015-government-policy-obesity-and-healthy-eating/2010-to-2015-government-policy-obesity-and-healthy-eating)

Vallianou N, Evangelopoulos A, Panagiotakos D. Associations of acute-phase reactants with metabolic syndrome in middle-aged overweight or obese people. Medical Science Monitor. 16(2): 56-60.

van Dam R, Feskens E. Coffee consumption and risk of type 2 diabetes mellitus. Lancet. 360: 1477–1478.

van Gaal L, Mertens I, De Block C. (2006). Mechanisms linking obesity with cardiovascular disease. Nature. 444: 875–880.

van Gaal LF, Mertens IL, De Block CE. (2006). Mechanisms linking obesity with cardiovascular disease. Nature. 444: 875–80

Vuong J, Qiu Y, La M, Clarke G, Swinkels D, Cembrowski G. (2014). Reference intervals of complete blood count constituents are highly correlated to waist circumference: should obese patients have their own “normal values?. American Journal of Hematology. 89(7): 671-677.

Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun, I, Shen C, (2014). Novel insights of dietary polyphenols and obesity. The Journal of nutritional biochemistry. 25(1): 1-18.

Wang X, Chai H, Wang Z, Lin P, Yao Q, Chen C. (2008). Serum amyloid A induces endothelial dysfunction in porcine coronary arteries and human coronary artery endothelial cells. American Journal of Physiological Heart Circulations Physiology. 6: 2399–2408.

Wang Y, Fofana B, Roy M, Ghose K, Yao X, Nixon M, Nair S, Nyomba G. (2015). Flaxseed lignan secoisolariciresinol diglucoside improves insulin sensitivity through upregulation of GLUT4 expression in diet-induced obese mice. Journal of Functional Foods. 18: 1-9.

Wardle J, Griffith J, Johnson F, Rapoport L. (200). Intentional weight control and food choice habits in a national representative sample of adults in the UK. International Journal of Obesity. 24: 534-540.

Wei M, Gaskill S, Haffner S, Stern M. (1997). Waist circumference as the best predictor of noninsulin dependent diabetes mellitus (NIDDM) compared to body mass index, waist/hip ratio and other anthropometric measurements in Mexican Americans–a 7-year prospective study. Obesity Research. 5: 16–23.

Welborn T, Dhaliwal S. (2007). Preferred clinical measures of central obesity for predicting mortality. European Journal of Clinical Nutrition. 61: 1373–1379.

Westerterp-Plantenga M. (2010). Green tea catechins, caffeine and body-weight regulation. Physiology & behaviour. 100(1): 42-46.

Winer DA, Winer S, Shen L, Wadia P, Yantha J, Paltser G, Tsui H, Wu P, Davidson M, Alonso MN, et al. (2011). B cells promote insulin resistance through modulation of T cells and production of pathogenic IgG antibodies. Nature Medicine. 17: 610–617.

Wing R, Hill J. (2001). Successful weight loss maintenance. Annual Review of Nutrition. 21: 323–341.

World Health Organisation Media Centre. (2013). Obesity and Overweight. Geneva, Switzerland. Available at: http://www.who.int/mediacentre/factsheets/fs311/en/

World Health Organization (WHO). (1999). Definition, Diagnosis, and Classification of Diabetes Mellitus and its complications Part 1: Diagnosis and Classification of Diabetes Mellitus. Provisional Report of a WHO Consultations Geneva: WHO. Available at: http://apps.who.int/iris/bitstream/10665/66040/1/WHO_NCD_NCS_99.2.pdf

Xu Y, Zhang M, Wu T, Dai S, Xu J, Zhou Z. (2015). The anti-obesity effect of green tea polysaccharides, polyphenols and caffeine in rats fed with a high-fat diet. Royal Society of Chemistry Food Function. 6: 296-303.

Yamakawa M, Yorifuji T, Inoue S, Kato T, Doi H. (2013). Breastfeeding and obesity among schoolchildren: a nationwide longitudinal survey in Japan. Journal of American Medical Association Paediatrics. 167: 919–25.

Yoon J, Baek S. (2005). Molecular targets of dietary polyphenols with anti-inflammatory

properties. Yonsei medical journal. 46: 585-596.

Yoshikawa M, Shimoda H, Nishida N, Takada M, Matsuda H. (2002). *Salacia reticulata* and its polyphenolic constituents with lipase inhibitory and lipolytic activities have mild antiobesity effects in rats. Journal of Nutrition. 132: 1819-1824.

Yu Z, Han S, Zhu G, Zhu C, Wang X, Cao X, Guo X. (2011). Birth weight and subsequent risk of obesity: a systematic review and meta-analysis. Obesity Reviews. 12(7): 525-542.

Yudkin J, Kumari M, Humphries S, MohamedAli V. (2000). Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? Atherosclerosis. 148(2). 209–214.

Yun J. (2010). Possible anti-obesity therapeutics from nature a review. Phytochemistry. 71: 1625-1641.

Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman J. (1994). Positional cloning of the mouse obese gene and its human homologue. Nature. 372(6505): 425-432.

Zhao Y, Sun R, You L, Gao C, Tian Z. (2003). Expression of leptin receptors and response to leptin stimulation of human natural killer cell lines. Biochemistry, Biophysics, Research, Community. 300: 247-252.

Zhao Y, Wang S, Mu M, Sheng J. (2012). Birth weight and overweight/obesity in adults: a meta-analysis. European Journal of Paediatrics. 171: 1737–1746.

Zhao Z, Li S, Liu G, Yan F, Ma X, Huang Z, Tian H. (2012). Body iron stores and heme-iron intake in relation to risk of type 2 diabetes: a systematic review and meta-analysis. PLoS One7: e41641.

Zou J, Lai B, Zheng M, Chen Q, Jiang S, Song A, Huang Z, Shi P, Tu X, Wang D, Lu L, Lin Z, Gao X. (2017). $CD4^+$ T cells memorize obesity and promote weight regain. Cellular & Molecular Immunology. 14: 1–10. doi: 10.1038/cmi.2017.36

ANNEXES

Annex 1: Ethics

MINISTERIO
DE ECONOMÍA
Y COMPETITIVIDAD

CSIC
CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS
COMITÉ DE ÉTICA
Subcomité de Bioética

INFORME DE EVALUACIÓN BIOÉTICA / BIOSEGURIDAD

El Subcomité de Bioética del CSIC ha evaluado los aspectos bioéticos y de bioseguridad de investigación propuesta, y según las condiciones expresadas en la misma, no ha encontrado ninguna objeción al respecto que pueda constituir un impedimento para su desarrollo. Y para que conste a los efectos oportunos, expide el presente informe.

Datos del Investigador principal

Nombre	MARCOS SÁNCHEZ, ASCENSIÓN
Centro / Instituto	Instituto de Ciencia y Tecnología de Alimentos y Nutrición (Madrid)
Teléfono	915492300 Ext. 297
Correo electrónico	amarcos@ictan.csic.es

Datos de la Investigación propuesta

Título	Evaluación de una bebida saludable para el control del peso corporal (Proyecto PRONAOS)
Convocatoria	PROYECTO PRONAOS 2011

Valoración

APROBADO

Este informe solo tiene validez para la investigación propuesta y en las condiciones en ella descritas. Cualquier cambio que afecte a las implicaciones bioéticas y/o de bioseguridad de la misma, invalida este informe y deberá ser puesto en conocimiento de este Subcomité para su valoración.

Madrid, 4 de Julio de 2012

P.O.

Jose Pío Beltrán Borter
Presidente del Subcomité de Bioética del CSIC

subcomitedebioetica@csic.es

C/ Serrano 113
28006 Madrid ESPAÑA
Telf.: 915681494
915681554

Annex 2: Ethics

Hospital Universitario Puerta de Hierro Majadahonda
SaludMadrid

Comunidad de Madrid

Dª. LOURDES CABRERA GARCÍA, SECRETARIA DEL COMITÉ ETICO DE INVESTIGACIÓN CLÍNICA DEL HOSPITAL UNIVERSITARIO PUERTA DE HIERRO MAJADAHONDA DE MADRID

C E R T I F I C A

Que dicho Comité ha evaluado el siguiente proyecto de investigación:

"EVALUACIÓN DE UNA BEBIDA SALUDABLE PARA EL CONTROL DEL PESO CORPORAL (PROYECTO PRONAOS)"

La investigadora principal es la Dra. Ascensión Marcos Sánchez. Se considera que su planteamiento global es correcto desde el punto de vista metodológico y ético. Acta nº 267 de fecha 27/06/11

En Madrid, a 9 de junio de 2011

Hospital Universitario Puerta de Hierro Majadahonda
SaludMadrid
Comunidad de Madrid
Comité Ético de Investigación Clínica

Fdo.: Dra. Cabrera García
Secretaria C.E.I.C.

ÁREA VI
C/ Joaquín Rodrigo, 2
28222 Majadahonda / Madrid
Tel.: 91 191 60 00
Fax: 91 373 05 35

Red H s H
Madrid
Hospitales sin Humo

Annex 3: PRONAOS project participant information sheet

www.proyectopronaos.es

INFORMATION FOR THE PARTICIPANTS

PROJECT TITLE: ASSESSMENT OF THE EFFECT OF A HEALTHY BEVERAGE ON BODY WEIGHT MANAGEMENT (PRONAOS PROJECT)

WHO CONDUCTS THE STUDY?

This work is being carried out by the **Immunonutrition Research Group** of the **Spanish National Research Council (CSIC).** The group is settled in the Institute of Food Science, Technology and Nutrition (**ICTAN**), at the University Complutense Campus (Ciudad Universitaria).

The study is part of the PRONAOS Project, an inniciative of the Spanish Ministry of Science and Innovation with the aim of encouraging academy and industry to collaborate in the development of novel functional food products for the prevention and management of overweight and obesity.

INTEREST OF THE STUDY

The main concern in relation to obesity lies in its association with diminished quality of life and with a higher risk of developing chronic diseases such as diabetes, cardiovascular disease, or even cancer.

Lifestyle habits, especially diet and physical activity, are essential factors in the onset of overweight and obesity, and consequently, they are also crucial for prevention and treatment.

WHAT IS THE AIM OF THE STUDY?

The aim is to assess whether the regular consumption of a functional beverage has an effect on body weight control. To help achieve it, we seek young volunteers (20-40 years old) with a body mass index in the range of overweight or obesity (≥24.5 kg/m^2) but otherwise healthy.

WHAT SHALL PARTICIPANTS DO?

Participants shall drink the beverage daily for 8 weeks. Then they will rest for 4-8 weeks (washout period) and then the treatment will be repeated for another 8 weeks. On the second phase of the treatment, the taste of the beverage will be slightly different, so as to estimate which flavor can be more successful later in the market.

Optimal beverage consumption is 1L/day (two bottles of 0.5 L). Our recommendation is to drink it during the day, preferably in the morning.

Participants will meet the researcher six times during the study: at the beginning, in the middle and at the end of each phase of treatment. On each meeting data will be collected from body weight and fat percentage, waist and hip circumference, and physical activity and dietary habits (see forms below).

On four of the six meetings (at the beginning and at the end of each phase) blood samples will be collected as well, for further analysis.

All data will be kept and analyzed anonymously. We guarantee your privacy and only you will have access to your personal individualized information upon request.

HOW TO PARTICIPATE?

Your participation in the study is completely voluntary and free.

If you are interested, you can contact us (Dr. Fátima Pérez de Heredia) by phone (915492300), from 10 to 14h, or by email (inmuno.nutri.ictan@gmail.com).

A personal interview will be then arranged to ask you some questions about personal data and medical history and to answer to all the questions you may have. If you fulfill all inclusion criteria (aged 20-40, body mass index ≥24.5 and being free of diseases that may interfere with the objectives of the study), you will be included in the study.

All appointments will take place at the Institute of Food Science, Technology and Nutrition (ICTAN, former *Instituto del Frío*), at the University Complutense Campus (Ciudad Universitaria, Madrid).

Participants who complete the study will receive 150€ as gratification.

IS IT SAFE TO PARTICIPATE?

The beverage does not contain alcohol, sugar, or any substance that can be considered toxic, or for which secondary effects have been described at the doses employed.

The beverage contains sources of caffeine, and therefore it could have a mild stimulatory effect. For this reason we recommend it to be consumed preferably during the morning or before 6 p.m.

It also contains sources of substances that can potentially interfere with iron absorption (although at the same level that tea and coffee do). Therefore, we recommend it not to be consumed during lunch and wait approximately an hour after having had lunch.

REGULATIONS FOR THE USE AND GUARANTEE OF PRIVACY OF THE INFORMATION OBTAINED

The project will be conducted in accordance with the ethical principles in the Declaration of Helsinki (52nd General Assembly, Edinburgh, Scotland, 2000), the Code of Good Clinical Practice, and with current legislation.

Sample processing

Biological samples (from blood extractions) will be kept in the ICTAN premises (CSIC), under the conditions and for the time required for analysis, in accordance with the objectives of the study, and will be conveniently destroyed once analyzed. Similarly, questionnaires will be stored in the ICTAN premises for the time required for analysis and will be destroyed after being analyzed.

Data processing

Each participant will be identified by a code, so as to preserve anonymity. All codified information will be also protected from non-authorized use by third parties. Data will not be shared with third persons and their use will be exclusively for scientific purposes, and no economic profit will be obtained through their exploitation.

The information resulting from the study will be considered strictly confidential for the parties involved; however, supervision by Health Authorities will be allowed when required, in accordance with the regulations included in the *Ley Orgánica de Protección de Datos (LOPD 15/1999)*. According to these regulations:

- All personal data subjected to processing cannot be used for any purpose incompatible with those for which data were initially collected.
- Further processing for historic, statistical or scientific purposes will not be considered incompatible.
- Data will not be kept in such a way that allows identification of the participants and they will be stored only for the time required to achieve the purposes for which they were collected.

Potential participants will be informed in an express, precise and unequivocal way of:

a) The storing and processing of personal data, of the aim(s) in obtaining these data, and of the person(s) who will have access to them.

b) The voluntary or compulsory nature of their responses to the questions that they will be asked.

c) The consequences of data collection and the refusal to facilitate them.

d) The possibility of having rights to access, correction, cancelation or opposition of personal data.

e) The identity and address of the researcher responsible for the treatment, or his/her representative instead.

The processing of personal data will require the unequivocal consent of the participants, save when the current legislation stipulates otherwise.

The consent to which the article refers can be revoked for a justified reason and when no retroactive effects apply.

The person responsible for the files and everyone who participates at any time in the collecting and processing of samples and data are bound by professional secrecy and shall observe it, even after termination of their relationship with the file holder or the person responsible instead.

Participants' access rights to personal information

1. Participants will have the right to request and freely obtain the information related to their personal data subjected to processing, to the origin of these data, and to any public communication that may result from them.
2. This information will be provided through simple visualization of data, or in written form, through copy, photocopy or telecopy, in a legible and understandable manner, free of codes that may require the use of specific devices.
3. The right of access to which this article refers can only be exercised at intervals no shorter than twelve months, unless the participant proves a legitimate interest, in which case he/she will be able to exercise his/her right earlier.

Annex 4: Informed consent PRONAOS project

www.proyectopronaos.es

PROJECT TITLE:

ASSESSMENT OF THE EFFECT OF A HEALTHY BEVERAGE ON BODY WEIGHT MANAGEMENT (PRONAOS PROJECT)

Informed consent

Participant's name:

Address:

Mr./Ms. ..... agrees to take part in the present study, which is included in the national PRONAOS Project, and is aimed at assessing the efficacy of a functional beverage in body weight management.

The volunteer has read and understood all verbal and written information provided by the researcher(s) in relation to his/her participation in the study. The volunteer has been allowed to ask and comment about this information and has been given satisfactory answers by the researcher(s). The volunteer knows that her/his participation is totally free and voluntary, and that she/he can drop out of the study at any time, without being obliged to justify it.

The volunteer expresses hereby her/his agreement on allowing Health Authorities to have access to medical data. The volunteer also agrees for the personal data collected during the study to be computerized (included those related to ethnicity, if necessary).

The project will be conducted in accordance with the ethical principles included in the Declaration of Helsinki (52nd General Assembly, Edinburgh, Scotland, 2000), the Code of Good Clinical Practice, and with current legislation, and under the regulations included in the *Ley Orgánica de Protección de Datos (LOPD 15/1999)*.

The volunteer will received a copy of this form once signed by all parties.

Signatures (compulsory):

Participant:	Researcher responsible:

Annex 5: Informed consent for blood extraction

MINISTERIO
DE CIENCIA
E INNOVACIÓN

CONSEJO SUPERIOR
DE INVESTIGACIONES
CIENTÍFICAS

Mr/Ms _____ with ID number _____, has been informed of the conditions of storage and custody of the blood samples that will be obtained as part of the study "Evaluation of the Efficacy of a treatment with a healthy drink on body weight and fat control and its effects on related health variables", included in the national Project Pronaos of the Spanish Ministry of Science and Innovation, and of the use that will be made of them. The responsibility of these samples belongs to the principal investigator, Professor Ascensión Marcos, with ID number 00265107D, and the Immunonutrition Research Group, and they will be kept at the premises of the Department of Metabolism and Nutrition of the Institute of Food Science and Technology and Nutrition (ICTAN), CSIC.

Madrid, _____, 2011.

Signature:

Annex 6: Personal data questionnaire

PARTICIPANT'S PERSONAL INFORMATION

CODE ☐☐☐☐ **(to be filled by the researcher)**

1. Gender: ☐ Male ☐ Female

2. Age: **3. Date of birth (dd/mm/yyyy):**

4. Where were you born?

☐ Spain → *Go to question 6*

☐ Another country:

5. When did you move to Spain (month and year)?

6. What is your weight (naked)? ☐☐☐ kg.

7. What is your height (barefoot)? ☐☐☐ cm.

8. What was your birth weight? ☐☐☐☐ g.

9. As an infant

☐ you were breastfed

☐ you were fed with infant formula

☐ you received both

10. How would you describe your health in the last 12 months?

☐ Very good

☐ Good

☐ Not too good/not too bad

☐ Bad

☐ Very bad

11. <u>In the last year</u>, have you been diagnosed by a doctor with any of the illnesses below?

☐ Infections (respiratory, digestive, etc.)

☐ Hypertension

☐ High cholesterol levels

☐ Overweight/Obesity

☐ Diabetes (high blood glucose)

☐ Heart disease

☐ Anemia

☐ Digestive disease

☐ Depression

☐ Other mental diseases

☐ None

12. Regarding medicament use <u>in the last 2 weeks</u>, have you taken any of the medicines below? If yes, were they prescribed by a doctor?

	Taken			Prescribed		
	Yes	No	Not sure	Yes	No	Not sure
Medicines for cold, flu, throat or chest diseases (except antibiotics)	❍	❍	❍	❍	❍	❍
Medicines for pain and/or temperature	❍	❍	❍	❍	❍	❍
Supplements (vitamins, minerals, etc.)	❍	❍	❍	❍	❍	❍
Laxatives	❍	❍	❍	❍	❍	❍
Antibiotics	❍	❍	❍	❍	❍	❍
Tranquilizers, sleeping pills	❍	❍	❍	❍	❍	❍
Medicines for hay fever or allergies	❍	❍	❍	❍	❍	❍
Medicines for diarrhea	❍	❍	❍	❍	❍	❍
Medicines for rheumatism, or anti-inflammatory	❍	❍	❍	❍	❍	❍
Medicines for heart alterations	❍	❍	❍	❍	❍	❍
Medicines for blood pressure	❍	❍	❍	❍	❍	❍
Medicines for digestive problems	❍	❍	❍	❍	❍	❍
Antidepressants, stimulants	❍	❍	❍	❍	❍	❍
Contraceptives	❍	❍	❍	❍	❍	❍
Slimming medicines	❍	❍	❍	❍	❍	❍
Medicines for lowering cholesterol levels	❍	❍	❍	❍	❍	❍
Medicines for diabetes treatment	❍	❍	❍	❍	❍	❍
Diuretics	❍	❍	❍	❍	❍	❍
Dermatologic medicines (for the skin)	❍	❍	❍	❍	❍	❍
Homeopathic remedies	❍	❍	❍	❍	❍	❍

13. Have you <u>ever</u> been diagnosed with HAY FEVER or other ALLERGIES?

□ No

□ Yes → How old were you? ☐☐ old → Do you get vaccinated? □ Yes □ No

I am allergic to: _____

14. Which of these options describes better your cigarette consumption?

□ I have never smoked or I quitted smoking more than three months ago → *go to question 16*

□ I have quitted smoking within the last three months → *go to question 16*

□ I smoke occasionally

□ I smoke regularly

15. How many cigarettes do you smoke on average?

☐☐ cigarettes/day on a weekday

☐☐ cigarettes/day on the weeken

16. How often do you drink these beverages?

	Never	Seldom	< 1 per week	1 per week	2-4 per week	5-6 per week	1 / day	2-4/day	5-6/day
Coffee	❍	❍	❍	❍	❍	❍	❍	❍	❍
Tea	❍	❍	❍	❍	❍	❍	❍	❍	❍
Other herbal teas (please specify)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Caffeinated soft drinks (with and without sugar)	❍	❍	❍	❍	❍	❍	❍	❍	❍
Energy drinks (Red Bull, Burn, etc.)	❍	❍	❍	❍	❍	❍	❍	❍	❍

17. Have you ever forced yourself vomiting after eating too much or feeling too full?

□ Yes

□ No

18. Have you got any concerns about losing control over the amount of food you eat?

□ Yes

□ No

19. Have you recently lost more than one stone (≈ 7 kg) in 3 months?

□ Yes

□ No

20. Do you think you are too fat although other people tell that you are slim?

□ Yes

□ No

21. Do you believe that food controls your life?

□ Yes

□ No

22. At present time, are you following any specific diet or taking any other measure to lose weight?

□ No, I believe that my weight is right.

□ No, but I believe I should lose some weight.

□ No, as I need to gain weight.

□ Yes

23. Which meals do you have during the day?

	Yes	No
Breakfast	☐	☐
Midmorning snack	☐	☐
Lunch	☐	☐
Afternoon snack	☐	☐
Dinner	☐	☐
Bed-time snack	☐	☐

24. How many glasses of water do you normally drink during the day?

_____ glasses

25. How many times a week do you normally eat these foods? *(Please choose only one option per row)*

	Never	< 1 per week	1 per week	2-4 per week	5-6 per week	Once a day	> Once a day
Fruits	❍	❍	❍	❍	❍	❍	❍
Soft drinks and other sugar-containing drinks	❍	❍	❍	❍	❍	❍	❍
Meat	❍	❍	❍	❍	❍	❍	❍
Grained-derived products (bread, rice, pasta, etc.)	❍	❍	❍	❍	❍	❍	❍
Pulses (beans, lenses, chickpeas, etc.)	❍	❍	❍	❍	❍	❍	❍
Vegetables	❍	❍	❍	❍	❍	❍	❍
Eggs	❍	❍	❍	❍	❍	❍	❍
Fish	❍	❍	❍	❍	❍	❍	❍
Cold and cured meats	❍	❍	❍	❍	❍	❍	❍
Sweets (candies, chocolate, etc.)	❍	❍	❍	❍	❍	❍	❍
Chicken	❍	❍	❍	❍	❍	❍	❍
Milk and dairy products	❍	❍	❍	❍	❍	❍	❍
Snacks (chips, crisps, etc.)	❍	❍	❍	❍	❍	❍	❍
Ready-made foods	❍	❍	❍	❍	❍	❍	❍
Fast food	❍	❍	❍	❍	❍	❍	❍

26. FOR WOMEN ONLY

How old were you when you had your first period? ☐☐ years old.

Date of your last period:

THANK YOU FOR YOUR COLLABORATION

3-DAY DIETARY RECORD

The dietary record is like a log where you will write down everything you eat and drink over 24 h. You will need to be particularly aware of common foods that usually go unnoticed, such as condiments, spices and salad seasoning, or the sugar added to beverages (tea, coffee, etc.).

A proper 3-day dietary record includes 2 working days plus a weekend or holiday day. For best results, you should write down your intake after each meal (breakfast, midmorning snack, lunch, afternoon snack, and dinner). When foods consist on elaborate meals (like soups, stews, etc.), try to specify the ingredients as precisely as possible (for instance, if you have had a stew, tell if it contained meat and what type of meat, what type of vegetables, and so on), and the rough amount you ate of each one.

On the firs column, you should write where you had your meal and how long it took; on the second column, write the foods you ate and the beverages you drank; and on the third column, the amount of each food.

It will often be necessary for you to specify the type of food – for instance, if you drink milk, you should write whether it is whole, skimmed or semi-skimmed; if you eat bread, whether it is baguette, tin bread, white bread, rye bread, etc.; and you should also specify the type of fats and oils you consume (olive, sunflower, corn…).

To express amounts, you can use standard home measures such as “spoon”, “glass”, “small/large plate”, etc. for uncountable foods. You can also use “slice”, “loaf”, and so on. For foods like fruits, vegetables or meat steaks, it is important to mention an approximate size (big, medium or small).

You will find an example 1-day record below.

Remember that the more precise and specific your answers are, the more useful the information becomes. That is why we recommend you not to wait long to record your meal after you eat.

THANK YOU FOR YOUR COLLABORATION!

DAY OF THE WEEK:		DATE:
	FOOD	**AMOUNT**
BREAKFAST		
Start:		
End:		
Place:		
MIDMORNING		
Start:		
End:		
Place:		
LUNCH		
Start:		
End:		
Place:		
AFTERNOON		
Start:		
End:		
Place:		
DINNER		
Start:		
End:		
Place:	-	

DAY OF THE WEEK:		DATE:
	FOOD	**AMOUNT**
BREAKFAST		
Start:		
End:		
Place:		
MIDMORNING		
Start:		
End:		
Place:		
LUNCH		
Start:		
End:		
Place:		
AFTERNOON		
Start:		
End:		
Place:home		
DINNER		
Start:		
End:		
Place:		

DAY OF THE WEEK:		DATE:
	FOOD	**AMOUNT**
BREAKFAST		
Start:		
End:		
Place:		
MIDMORNING		
Start:		
End:		
Place:		
LUNCH		
Start:		
End:		
Place:		
AFTERNOON		
Start:		
End:		
Place:		
DINNER		
Start:		
End:		
Place:		

3-DAY DIETARY RECORD

The dietary record is like a log where you will write down everything you eat and drink over 24 h. You will need to be particularly aware of common foods that usually go unnoticed, such as condiments, spices and salad seasoning, or the sugar added to beverages (tea, coffee, etc.).

A proper 3-day dietary record includes 2 working days plus a weekend or holiday day. For best results, you should write down your intake after each meal (breakfast, midmorning snack, lunch, afternoon snack, and dinner). When foods consist on elaborate meals (like soups, stews, etc.), try to specify the ingredients as precisely as possible (for instance, if you have had a stew, tell if it contained meat and what type of meat, what type of vegetables, and so on), and the rough amount you ate of each one.

On the firs column, you should write where you had your meal and how long it took; on the second column, write the foods you ate and the beverages you drank; and on the third column, the amount of each food.

It will often be necessary for you to specify the type of food – for instance, if you drink milk, you should write whether it is whole, skimmed or semi-skimmed; if you eat bread, whether it is baguette, tin bread, white bread, rye bread, etc.; and you should also specify the type of fats and oils you consume (olive, sunflower, corn…).

To express amounts, you can use standard home measures such as “spoon”, “glass”, “small/large plate”, etc. for uncountable foods. You can also use “slice”, “loaf”, and so on. For foods like fruits, vegetables or meat steaks, it is important to mention an approximate size (big, medium or small).

You will find an example 1-day record below.

Remember that the more precise and specific your answers are, the more useful the information becomes. That is why we recommend you not to wait long to record your meal after you eat.

THANK YOU FOR YOUR COLLABORATION!

DAY OF THE WEEK: Monday		DATE: June 13th 2011
	FOOD	**AMOUNT**
BREAKFAST		
Start: 8.00	White coffee:	1 cup
End: 8.20	- Semiskimmed milk	½ glass
Place: home	- Sugar	1 spoon
	Toasts (tin white loaf):	2 slices
	- Butter	2 spoons
	- Jam	2 spoons
MIDMORNING		
Start: 10.15	Orange juice	1 glass
End: 10.45	Cheese roll: - white bread	1 baguette
Place: canteen	- Gouda cheese	1 slice
LUNCH		
Start: 14.30	Lettuce and tomato salad	1 small plate
End: 15.30	- Olive oil and vinager	1 spoon + ½ spoon
Place: canteen	Roast chicken:	1 chicken leg
	- Potatoes	1 medium
	- Onions, tomato	½ small plate
	Beer	1 glass
	Orange	1 medium
AFTERNOON		
Start:	Nothing	
End:		
Place:		
DINNER		
Start:	Courgette soup (courgettes, potatoes, olive oil)	1 plate
End:	Omelette	2 eggs
Place: canteen	- Olive oil	1 spoon
	Apple	1 big
	Semiskimmed milk	1 glass

Annex 9: Correlation coefficients between estimated caffeine consumption and body composition-related variables in total sample and according to gender.

	All					Females					Males				
	BMI	**Body fat (%)**	**Waist (cm)**	**WHR**	**WHeR**	**BMI**	**Body fat (%)**	**Waist (cm)**	**WHR**	**WHeR**	**BMI**	**Body fat (%)**	**Waist (cm)**	**WHR**	**WHeR**
Total caffeine (mg/day)	0.008	0.030	0.110	0.036	0.029	0.073	0.054	0.301	0.360	0.160	-0.031	0.110	0.074	-0.014	-0.052
Caffeine from tea and coffee (mg/day)	-0.092	-0.002	0.067	0.71	-0.040	-0.027	-0.018	0.164	0.122	0.049	-0.130	0.075	0.056	0.001	-0.101
Caffeine from caffeinated and energy drinks (mg/day)	-0.009	-0.017	0.033	0.001	0.011	0.102	0.200	0.274	0.063	0.147	-0.063	0.002	-0.061	-0.114	-0.067

Pearson correlation used. Significance set at P< 0.05. BMI: body mass index; WHR: waist-to-hip ratio; WHeR: waist-to-height ratio.

Annex 10: Effect of the treatments in all participants.

	All						
	Placebo		Functional				
	Baseline	Endpoint	Baseline	Endpoint	P_d	P_t	P_i
Basophil (x10^3/mm^3)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.1-0.1)	**0.009**	0.636	0.350
Leptin (ng/ml)	4.9±3.8	7.4±6.5	6.4±6.2	7.6±6.9	**0.006**	0.201	0.349
VCAM1 (ng/ml)	119.8±32.1	106.8±25.9	119.9±27.0	111.5±25.7	**0.001**	0.525	0.386
ICAM1 (ng/ml)	93.6 (80.1-113.0)	91.4 (69.9-120.9)	93.5 (76.5-111.7)	91.0 (73.0-123.2)	**0.039**	0.865	0.691
NEFA (mmol/L)	0.02±0.01	0.03±0.01	0.03±0.01	0.03±0.01	**0.004**	0.835	0.349

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and final values compared by two-way ANOVA. Significance at *P < 0.05. VCAM-1: vascular cell adhesion protein 1; ICAM-1: Intracellular adhesion molecule; NEFA: Non-esterified fatty acids

Annex 10: Effect of the treatments in female and male participants.

	Female							Male						
	Placebo		Functional		P_d	P_t	P_i	Placebo		Functional		P_d	P_t	P_i
	Baseline	Endpoint	Baseline	Endpoint				Baseline	Endpoint	Baseline	Endpoint			
WBC (x10³/mm³)	6.6 (5.4 –7.6)	6.4 (5.5-7.3)	6.7 (5.2–7.6)	6.2 (5.7– 7.5)	NS	NS	NS	6.2 (5.3-7.4)	6.3 (5.4-7.5)	6.0 (5.0-6.9)	5.7 (4.8-6.3)	NS	**0.047**	NS
Lyphocyte (x10³/mm³)	2.2 (1.9– 2.6)	2.3 (1.9– 2.6)	2.4 (2.0–2.6)	2.4 (2.1-2.7)	NS	NS	NS	2.2 (1.9-2.6)	2.3 (1.9-2.8)	2.2 (1.8-2.5)	2.0 (0.1-0.2)	NS	**0.042**	NS
Eosinphil (x10³/mm³)	0.1 (0.1– 0.2)	0.2 (0.1– 0.3)	0.2 (0.1–0.3)	0.2 (0.1– 0.2)	NS	NS	NS	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.2 (0.1-0.2)	0.2 (0.1-0.2)	NS	**0.005**	NS
Basophil (x10³/mm³)	0.0 (0.0– 0.1)	0.0 (0.0-0.1)	0.0 (0.0–0.1)	0.0 (0.0 -0.1)	NS	NS	NS	0.0 (0.0-0.1)	0.0 (0.0-0.1)	0.0 (0.0-0.0)	0.0 (0.0-0.0)	NS	**0.000**	NS
Leptin (ng/ml)	8.0±4.0	12.9±6.9	10.5±7.5	12.9±7.3	NS	**0.002**	NS	3.0±2.0	3.7±2.4	3.6±2.8	4.0±3.5	NS	NS	NS
Grehlin (pg/ml)	209.2±147.2	463.7±186.9	198.3±136.4	437.0±200.9	NS	**0.000**	NS	440.4±925.1	329.2±356.9	395.6±884.3	932.5±3701.3	NS	NS	NS
IL-1β (pg/ml)	1.0 (0.5-1.5)	0.7 (0.5-1.1)	0.2 (0.2-0.5)	1.0 (0.3-2.0)	NS	NS	**0.046**	4.4 (1.2-7.7)	1.1 (0.9-1.5)	1.0 (0.5-6.7)	0.9 (0.8-1.2)	NS	**0.013**	NS
TNF-α (pg/ml)	1.4 (0.7-4.4)	1.1 (0.4-20.8)	1.4 (0.8-3.6)	1.1 (0.8-5.6)	NS	**0.036**	NS	2.9 (1.2-7.2)	3.9 (2.4-6.6)	4.5 (0.6-18.4)	3.1 (1.3-9.7)	NS	NS	NS
VCAM1 (ng/ml)	114.2±27.3	107.2±28.1	116.3±25.2	108.2±21.6	NS	NS	NS	123.3±34.5	106.5±246.2	121.0±28.3	113.7±28.2	NS	**0.005**	NS
ICAM1 (ng/ml)	82.7 (68.0-98.9	89.4 (64.9-97.6)	87.9 (73.1-101.1)	91.1 (70.4-105.9)	NS	NS	NS	102.6 (82.1-118.7)	93.6 (70.7-142.0)	97.6 (81.5-116.7)	90.7 (77.5-169.1)	NS	**0.039**	NS
NEFA (mmol/L)	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	NS	NS	NS	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.01	**0.006**	NS	NS
Glucose (mmol/L)	4.8 (4.4-5.2)	4.4 (4.0-4.8)	4.8 (4.6-5.1)	4.3 (3.9-4.8)	**0.001**	NS	NS	5.1 (4.8-5.2)	5.0 (4.8-5.4)	5.0 (4.5-5.3	5.1 (4.7-5.4)	**0.011**	NS	NS
Cholesterol (mmol/L)	9.7±2.1	9.0±2.1	9.5±1.9	8.8±1.7	**0.041**	NS	NS	9.3±1.9	9.8±1.7	9.3±1.8	9.8±1.6	NS	NS	NS
Iron (mg/dl)	68.0 (43.0-97.0)	57.5 (41.0-88.0)	72.0 (53.5-99.5)	61.0 (38.0-97.0)	NS	NS	NS	98.0 (76.0-117.0)	96.0 (74.0-118.5)	87.0 (70.0-106.0)	86.0 (70.0-108.0)	NS	**0.044**	NS
Transferrin (mg/dl)	260.1±47.8	243.18±48.7	261.1±52.8	244.0±51.2	**0.048**	NS	NS	242.3±43.3	257.5±35.1	242.6±41.1	256.7±32.7	**0.008**	NS	NS
CD4⁺RA⁺ (Cell/mm³)	443.4±196.5	486.1±216.4	534.8±172.9	537.0±174.7	NS	**0.038**	NS	431.7±192.6	461.6±197.5	462.4±221.8	439.8±237.7	NS	NS	NS
CD4⁺RO⁺ (Cell/mm³)	540.0 (336.0-707.1)	553.9 (384.3-713.6)	404.1 (277.2-519.3)	389.1 (325.4-624.7)	NS	**0.003**	NS	403.4 (276.9-520.0)	429.7 (294.1-584.8)	421.5 (311.8-597.9)	426.3 (325.8-521.4)	NS	NS	NS

Data presented mean ± SD, or median (Q1, Q3), depending on normality. Baseline and final values compared by two-way ANOVA. Significance at *P < 0.05. WBC: white blood cell; IL-β: interleukin-1 Beta; TNF-α: tumor necrosis factor alpha; VCAM-1: vascular cell adhesion protein 1; ICAM-1: Intracellular adhesion molecule; NEFA: Non-esterified fatty acids; Lymphocyte populations are designated by their cell membrane markers, and defined by the anchor marker, which appears in first place of the subset name.