ARE ANTHROPOMETRIC BIOMARKERS, NUTRIENT INTAKE AND BLOOD FATTY ACID COMPOSITION ASSOCIATED WITH THE ELECTRICAL ACTIVITY OF THE HEART IN A SAMPLE POPULATION OF HEALTHY WOMEN?

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ABSTRACT

Cardiovascular disease (CVD) is a significant global burden. Obesity is considered a major causative factor in its development, and a poor diet with a positive energy balance is the main cause of obesity. Fatty acids (FAs) are a vital aspect of the human diet, and polyunsaturated fatty acids (PUFAs), particularly those from the n-3 family, are considered to bestow the most beneficial effects in the human body. This study was designed to investigate whether anthropometric biomarkers, nutrient intake and blood FA composition were associated with the electrical activity of the heart in a sample population of women, to support a link between FAs and cardiac function. Laboratory testing on 23 participants involved gathering anthropometric data, electrocardiograms (ECGs), and capillary blood samples, plus 4-day food diaries. Dietary analysis was used to quantify intakes of FAs and other macronutrients, ECG parameters were measured, and FA methylation and gas chromatography were used to determine blood FA levels. Associations between all variables were tested for statistical significance. There were some positive associations found between markers of obesity and disruption of the cardiac conduction system. Different measures of total saturated fatty acid (SFA) were associated with negative effects on the electrical activity of the heart, although some individual SFAs were seen to correlate with blood pressure in ways that suggested positive effects. The ratio of n-6:n-3 PUFAs was positively associated with the area under the curve (AUC) of the QRS complex and the R wave amplitude of the ECG - both measures of ventricular depolarisation - suggesting that a high n-6:n-3 PUFA ratio is detrimental to ventricular function. A negative relationship was found between docosahexaenoic acid (DHA), an important individual n-3 PUFA, and the AUC of the QRS, suggesting a beneficial effect of DHA on ventricular depolarisation. Although causation cannot be confirmed, these results are likely due to the pivotal role that PUFAs play in cell membranes, and particularly in the efficient function of cardiac ion channels, but also in the reduction of chronic inflammation. PUFAs of both the n-6 and n-3 families are essential nutrients and as such must be taken in in sufficient quantities in the diet. Intakes of n-3 are low in many countries and public health messages aimed at reducing CVD prevalence must focus on reducing SFA consumption and increasing n-3 PUFA intake so that the ratio of n-6:n-3 is, at most, 5:1.

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ABBREVIATIONS

AA	Arachidonic acid
ALA	Alpha-linolenic acid
AP	Action potential
ARP	Absolute refractory period
AUC	Area under the curve
AVN	Atrioventricular node
BHT	Butylated hydroxy toluene
BMI	Body mass index
CAD	Coronary artery disease
CLA	Conjugated linoleic acid
COX	Cyclooxygenase
CVD	Cardiovascular disease
DBP	Diastolic blood pressure
DGLA	Dihomo-gamma-linoleic acid
DHA	Docosahexaenoic acid
DoH	Department of Health
ECG	Electrocardiogram
EDA	Eicosadienoic acid
EFA	Essential fatty acid
EPA	Eicosapentaenoic acid
FAME	Fatty acid methyl esters
FA	Fatty acid
FAO	Food and Agriculture Organization (of the United Nations)
GC-FID	Gas chromatography - flame ionisation detection
GLA	Gamma-linoleic acid
HDL	High-density lipoprotein
I-TFA	Industrially-produced trans fatty acid
LA	Linoleic acid
LC-PUFA	Long-chain polyunsaturated fatty acid
LC-SFA	Long-chain saturated fatty acid
LDL	Low-density lipoprotein
LOX	Lipoxygenase
MAP	Mean arterial pressure

MC-SFA	Medium-chain saturated fatty acid
MUFA	Monounsaturated fatty acid
n-3	Omega-3 (family of PUFA)
n-6	Omega-6 (family of PUFA)
NA	Nervonic acid
OFN	Oxygen-free nitrogen
PUFA	Polyunsaturated fatty acid
R-TFA	Ruminant trans fatty acid
SACN	Scientific Advisory Committee on Nutrition
SAN	Sinoatrial node
SBP	Systolic blood pressure
SC-SFA	Short-chain saturated fatty acid
SD	Standard deviation
SFA	Saturated fatty acid
SR	Sarcoplasmic reticulum
TFA	Trans fatty acid
VA	Vaccenic acid
VLC-SFA	Very-long-chain saturated fatty acid
VLDL	Very low-density lipoprotein
WHO	World Health Organisation

INTRODUCTION

The burden of cardiovascular disease

Cardiovascular disease (CVD) affects more than 500 million people worldwide and is the leading cause of death across the globe, with 18.6 million deaths attributed to it in 2019 (Roth *et al.,* 2020). Consisting of a number of conditions that affect the heart and the blood vessels, it can take many forms, the most common of these being coronary artery disease (CAD). CAD occurs as a result of atherosclerotic plaques building up in the arteries that supply blood to the heart. This reduces blood supply, and therefore oxygen supply, to the myocardium, reducing efficiency of these cells, which can cause arrhythmia and ultimately heart failure. These arterial blockages can also lead to ruptures or cause blood flow to stop entirely, resulting in a heart attack. Other forms of CVD include stroke and transient ischaemic attack, peripheral arterial disease, aortic disease and arrhythmias (Roth *et al.,* 2020).

CVD is a multifactorial disease, with modifiable factors such as tobacco use, hypertension, hyperglycaemia, inactivity, high amounts of circulating low-density lipoprotein (LDL) cholesterol, alcohol consumption and obesity all playing their part in its prevalence (Roth *et al.,* 2020). This is a major issue for modern society since around 40% of the world's population is now thought to have a body mass index (BMI) of over 25 and is therefore classed as overweight or obese (WHO, 2021). In fact, the risk of heart failure increases by around 5% and 7% in men and women, respectively, with each single unit that BMI increases (Kenchaiah *et al.,* 2002). This is due mainly to the insulin resistance, endothelial dysfunction and increased inflammation that are associated with increased adiposity (Couillard *et al.,* 2005; Engin, 2017; Grundy, 2016; Rocha & Libby, 2009).

Diet and CVD

It is widely accepted that an energy-rich diet, alongside a lack of physical activity, resulting in a positive energy balance, is the main reason for the increased levels of obesity that we see around the world nowadays. A study in children showed that those eating more junk food, fewer fruit and vegetables, having more than 2 hours screen time per day, and spending less time engaging in physical activity were more likely to be overweight or obese (Ishaque *et al.,* 2012). It has also been recognised that obese children and more likely to grow up to be obese adults (Parsons *et al.,* 1999). Health education and improvements to lifestyle and diet from a young age could therefore be key in reducing levels of obesity, and the associated risk of CVD.

However, reducing weight is not the only way in which we can use diet to affect the risk of CVD. Independent of obesity-status, specific components of any individual diet can increase or

decrease the risk of CVD. Consuming too much salt can lead to hypertension (Grillo *et al.,* 2019); consuming too much saturated and trans-fat can increase LDL levels in the blood (Iqbal, 2014; Mensink & WHO, 2016); eating too much added sugar can increase blood pressure, triglyceride levels, cholesterol and inflammation (Aeberli *et al.,* 2011; Brown *et al.,* 2011; Fried & Rao, 2003; Welsh *et al.,* 2010). Regrettably, salt, sugar and unhealthy fats are all popular components of a modern-day diet, as processed food manufacturers use them to create flavourful products that consumers will crave and even become addicted to (Onaolapo & Onaolapo, 2018; Popkin *et al.,* 2012). Making a conscious choice to reduce intake of these foods can therefore reduce CVD risk in any individual, obese or not.

On the other hand, there are also a number of compounds that we can eat more of in order to positively affect heart health. By increasing our intake of foods such as fruits, vegetables, fish and seafood, wholegrains and nuts, we introduce a wide range of beneficial compounds and molecules into the body that bestow cardioprotective effects. These compounds, including antioxidant vitamins and minerals, phytochemicals, fibre and particular fatty acids (FAs), have all been shown to benefit heart health by such means as reducing inflammation, cholesterol and blood pressure, and protecting cells from damage (Mozaffarian *et al*., 2011). The fatty acids which are widely regarded as most beneficial to cardiovascular health are polyunsaturated FAs (PUFAs), in particular those of the omega-3 (n-3) family (Visioli & Poli, 2020). Unfortunately, intakes of PUFAs have reduced significantly over the past 150 or so years, due to the industrial and agricultural revolutions. Global diets have seen massive transformations away from natural, unprocessed foodstuffs such as fruits, vegetables, fish and lean meat, towards a greater consumption of highly processed items, with a focus on carbohydrates and saturated fats, and as a result, CVD has been on the increase (Simopoulos, 2006).

Fatty acids

Fat is an important aspect of the human diet, and the Committee on Medical Aspect of Food Policy advises that up to 35% of an adult's daily energy should be derived from it (DoH, 1991). Fat has had its fair share of bad publicity in recent years, as obesity levels have risen and diets are scrutinised, leaving people confused as to what they should and shouldn't be consuming. Research into FAs and their range of both positive and negative effects on the body is extensive and as a result is contentious and can be contradictory. The problem arises from the diversity of the molecules which fall under the 'fatty acid' umbrella, and our understanding of the function of each of these molecules once inside the body.

Dietary FAs tend to be ingested as triglycerides, which consist of three FA units bound to one molecule of glycerol, hydrolysed in the body to give free FA molecules, plus glycerol (Figure 1). Primarily, they are used as a source of cellular fuel as they are catabolised into carbon dioxide and water via the process of beta-oxidation, followed by the citric acid cycle and oxidative phosphorylation pathways, with energy being produced in the form of adenosine triphosphate (ATP). This process yields more than double the amount of energy for the cells than the breakdown of either carbohydrates or protein (SACN, 2011). We also know that FAs are important structural elements of cell membranes, in the form of phospholipids, and in this context are instrumental in the maintenance of cell membrane fluidity, which is essential for efficient cell function and growth (De Carvalho & Caramujo, 2018). Furthermore, they are significant precursors to molecules used for cell signalling (e.g. eicosanoids) (Berland *et al.,* 2016) and in the regulation of gene transcription (Georgiadi & Kersten, 2012). Not only this, but fats are vital in ensuring the absorption, storage and transportation via the lymphatic system, of the lipophilic vitamins A, D, E and K.

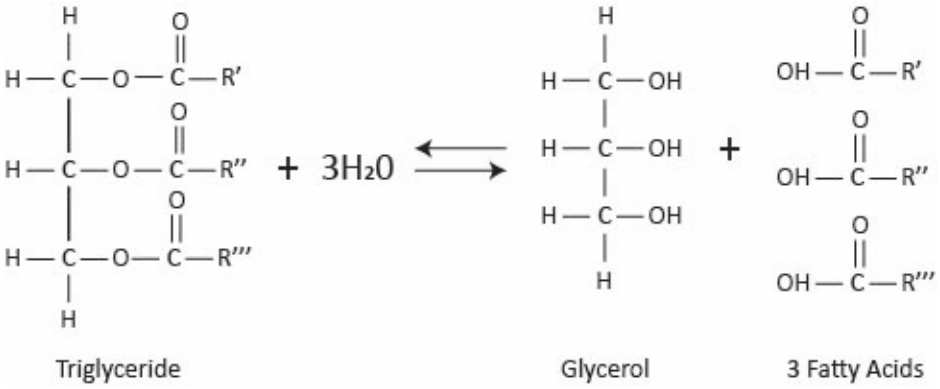

Figure 1. Hydrolysis of a triglyceride to give free fatty acids and glycerol. Adapted from Russell *et al.,* (2008)

Fatty acids are made up of a hydrocarbon chain usually consisting of between four and 28 carbon atoms, with a carboxyl group (-COOH) bonded to one end, and a methyl group (-CH3) at the other. They are named short-, medium-, long- or very-long-chain FAs depending on the number of carbons in the chain and can be saturated or unsaturated. In a saturated fatty acid (SFA) there are no double bonds between the carbon atoms, so the structure is stable, rigid and stackable, and as a result SFAs are usually solid at room temperature (Figure 2). Unsaturated FAs can be further divided into two classes: monounsaturated FAs (MUFAs), which contain one double bond (Figure 3), and polyunsaturated FAs (PUFAs), which contain two or more double bonds between carbon atoms (Figure 4). FAs are given both common names and numeric names, with the latter being dependent upon the number of carbon atoms and double bonds within the chain, and the

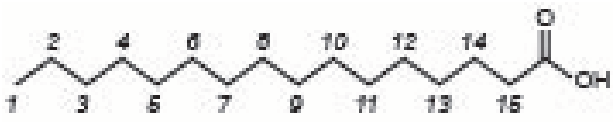

Figure 2. Simple structure of the saturated fatty acid C18:0 (palmitic acid), with carbon atoms numbered. Adapted from Yoon *et al.,* 2018.

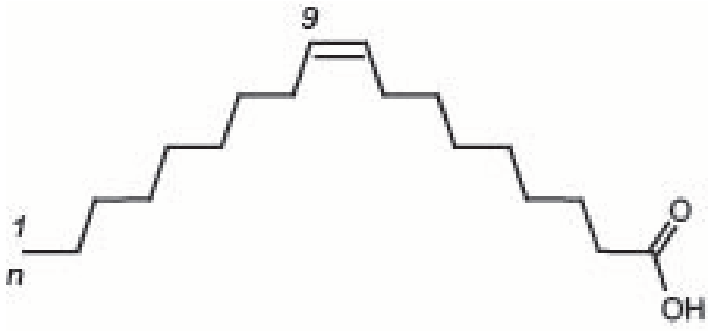

Figure 3. Structure of the monounsaturated fatty acid, oleic acid (C18:1n-9), showing the position of the 'n' carbon in relation to the double bond. Adapted from Yoon *et al.,* 2018.

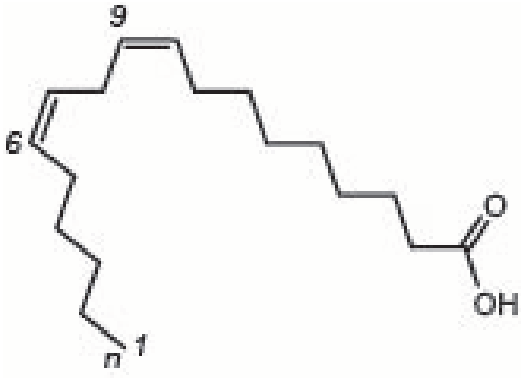

Figure 4. Structure of the polyunsaturated fatty acid, linoleic acid (C18:2n-6), showing the position of the 'n' carbon in relation to the 2 double bonds. Adapted from Yoon *et al.,* 2018.

position of the double bond closest to the methyl end of the molecule (also known as the 'omega' or 'n' end). For example, the PUFA linoleic acid is given the numeric name C18:2n-6 as it comprises 18 carbon atoms, and has two double bonds, with the first one situated at the sixth carbon from the molecule's omega end.

Fatty acids have become the subject of much debate as research unfolds into the role that diet can play in chronic disease development. In order to understand this debate, it is important to recognise how these various types of FA differ from one another.

Saturated fatty acids

Dietary sources of SFAs are predominantly meat and dairy products, eggs, coconut oil and processed foods. The relationship between SFA intake and cardiovascular problems is highly examined, yet still not completely understood due to the heterogeneous nature of SFA molecules. What all SFAs do have in common is a carbon chain where all available bonds are taken by a hydrogen atom, making them less easily oxidised in the body than unsaturated FAs. As well as being generally regarded as pro-inflammatory molecules (Zhou *et al.*, 2020), studies have shown a tendency for dietary SFAs to have a negative impact on the blood lipid profile by interfering with the activity of hepatic LDL receptors, which play an integral role in the removal of harmful LDL from the bloodstream (Dietschy, 1998). As previously stated, elevated LDL levels in the blood are a known risk factor for CVD. The highest risk of CVD comes from the long-chain SFAs (LC-SFAs), particularly palmitic acid (Fretts *et al.,* 2014), which is found in ultra-processed foods such as baked goods, ice creams and ready-meals (Houston, 2018), as these are often produced using high amounts of crude palm oil and palm kernel oil. This type of oil is favoured in these processes as it affords the manufacture of a low-cost product with a long shelf life (Steele *et al.,* 2021).

A study by Zhuang and colleagues (2019) has shown that as the intake of SFA increases by 5%, so the risk of overall mortality increases by 8%. It is therefore commonly advised to avoid highly processed foods to ensure that daily intake of SFA is no more than 10% of total daily energy, which equates to around 30g for an adult male, and no more than 24g for an adult female (DoH, 1991). Numerous studies, including a recent systematic review of 15 randomised controlled trials, have concluded that reducing overall SFA intake, and instead taking in the equivalent energy in the form of PUFAs or carbohydrates is associated with a decreased risk of developing CVD (Chen *et al.,* 2016; Jakobsen *et al.,* 2009). Additional research has found that the larger the reduction in SFA consumption, the greater the risk reduction (Hooper *et al.,* 2020).

Monounsaturated fatty acids

The most common MUFAs tend to be long-chain varieties (>16 carbon atoms), and they all contain a single double bond within the carbon chain. MUFAs can be endogenously synthesised, so are not essential in the diet, but dietary sources include meat and dairy products, nuts, and fatty fruits such as olives and avocados, which are all prime features of a traditional Mediterranean diet (Trichopoulou *et al*., 1993). The Mediterranean diet is widely considered to be a cardioprotective regime, with rates of CVD markedly lower in countries of Southern Europe, compared to those in Northern Europe, and this is widely attributed to the high MUFA content of

this diet (Dontas *et al*., 2007; Pitsavos *et al*., 2003). Olive oil, which can account for around 20% of total calorie intake in Greeks (Moschandreas & Kafatos, 1999), contains around 70% oleic acid (Beltrán *et al*., 2004), the predominant MUFA found in most diets.

It is not surprising therefore, that much research has been carried out into the causal relationship between dietary MUFA intake and the risk factors for CVD. MUFAs are thought to have anti-inflammatory properties (Ravaut *et al.,* 2020) and the capacity to affect the amounts of circulating LDL cholesterol (Cao *et al*., 2022), both of which are relevant to the development of cardiovascular issues. The results of these numerous studies are often inconsistent, however. Many studies deduce that the recognised relationship between the Mediterranean diet and the lower incidence of CVD and its risk factors must be directly attributable to the high levels of MUFAs, and the high ratio of MUFA to SFA (Pitsavos *et al.,* 2003; Teres *et al.,* 2008). However, other research contradicts this (Morin *et al.,* 2018), implying that many of the cardioprotective effects of a Mediterranean diet could come from different aspects, such as the concomitant high PUFA intake, and the higher levels of healthful polyphenolic compounds as compared to a more 'Western' diet (Visioli *et al.,* 2018: Dontas *et al.,* 2007). Guasch-Ferré *et al.* (2019) went on to highlight that MUFAs come from a variety of dietary sources and the beneficial MUFAs are those from plant origins, rather than those derived from meat, and that further study should take this into account.

The above-mentioned MUFA molecules are all characterised as *cis*-fats because the hydrogen atoms at the double bond are both on the same side of the carbon chain. Another, less commonly occurring unsaturated fat is the *trans*-fat, so-called because the hydrogens at the double bond sit on opposite sides of the chain (Figure 5.)

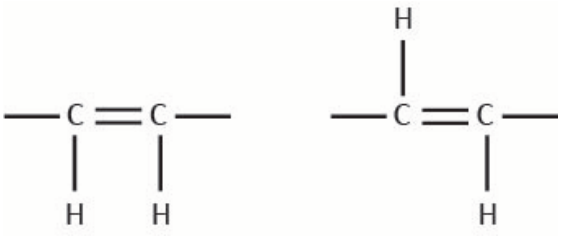

Figure 5. Diagram showing the arrangement of hydrogen atoms around the double bond in a *cis*-fatty acid (left) and a *trans*-fatty acid (right). Adapted from Russell *et al.,* 2008.

Trans fatty acids

Trans fatty acids (TFAs) are typically only found in small amounts in food products. They can be divided into two groups: R-TFA (ruminant TFA) and I-TFA (industrially-produced TFA). R-TFAs are

synthesised by the process of fermentation in the stomachs of ruminant animals such as sheep, goats and cows, and so are naturally occurring in dairy products. I-TFA are industrially synthesised in the production of food items such as cakes, biscuits and margarine (Dhaka *et al.,* 2011; Kuhnt *et al.,* 2011). This synthesis occurs as unsaturated fats are partially hydrogenated in order to increase the shelf-life of the product. As hydrogen is added, it bonds to the carbon chain in a *trans*-configuration, giving the molecule a straighter structure than a *cis*-unsaturated fat, therefore allowing the molecules to pack more tightly together. The result is a product that will spoil more slowly as it is less susceptible to oxidation (Kuhnt *et al*., 2011).

There is no recognised function that TFAs play within the human body (Brouwer *et al.,* 2013), and the negative health consequences of high I-TFA intake have been documented for decades, with a particular focus on the increased risk of developing CVD (Mensink & Katan, 1990; Oomen *et al*., 2001; Willett *et al.,* 1993). I-TFAs most damaging outcome is to increase the amount of LDL circulating in the blood, while simultaneously reducing the amount of 'good' high density lipoprotein (HDL) cholesterol (Mensink & Katan, 1990). Less negativity surrounds R-TFAs and their effects on human health (Stender *et al.,* 2008). It is understandable, therefore, that the World Health Organisation (WHO) launched a strategy to ensure that industrially-manufactured TFAs are completely eliminated from food products by 2023 (WHO, 2018).

Polyunsaturated fatty acids

PUFAs are so called because they contain two or more *cis* double bonds in the carbon chain, which affect the conformation of the molecule, creating a bend, or kink, in the backbone, as seen in Figure 4. The result of this is that they lack the same rigidity as SFAs, and therefore PUFAs bestow more biological benefits than SFAs do, especially when it comes to the body's inflammatory response, resistance to insulin and in particular, plasma membrane fluidity (Calder, 2017; Hąc-Wydro & Wydro, 2007; Hulbert *et al.,* 2005).

PUFAs are categorised dependent on where the first bond from the methyl, or omega, end of the molecule is situated. If the bond occurs at the third carbon from the omega end, the molecule is named an omega-3 PUFA, which is also written as 'n-3'. If the first bond is at the sixth carbon from the omega end, it is an n-6 PUFA, and so on. Most PUFAs are n-3 or n-6, although n-7 and n-9 forms also exist.

Whilst most FAs are named non-essential fatty acids, as they can be synthesised endogenously by way of elongation and desaturation reactions, the term 'essential fatty acid' (EFA) is given to a category of PUFA that cannot be produced in the mammalian body as the delta-12 and delta-15

desaturase enzymes required to manufacture them from other FAs are lacking (Lee *et al.,* 2016). These FAs must therefore be taken in via the food that we eat. Both n-3 and n-6 PUFAs are classed as EFAs, and the most commonly ingested are the n-3, alpha-linolenic acid (ALA), and the n-6, linoleic acid (LA) (Eilander *et al.,* 2015). ALA is an 18-carbon PUFA with three *cis* double bonds, and so is written as 18:3n-3. Dietary sources are predominantly plants and oils, such as flaxseed, canola, hemp and soybean oils, walnuts and leafy green vegetables (Hunter, 1990). LA consists of an 18-carbon chain with two *cis* double bonds (18:2n-6) and is primarily found in oils such as corn, sunflower and safflower oil (Bézard *et al*., 1994).

PUFA metabolism

One fate of these PUFAs upon ingestion is their metabolism, primarily in the endoplasmic reticulum of the liver, into longer-chain PUFAs (LC-PUFAs) that are important for human health (Arterburn *et al.,* 2006). This happens by way of enzymatic cascades involving desaturase and elongase enzymes which add into the carbon chain a double bond or an additional pair of carbon atoms, respectively. Two significant LC-PUFAs of which ALA is the precursor are eicosapentaenoic acid (EPA) (20:5n-3) and docosahexaenoic acid (DHA) (22:6n-3). The conversion rate is slow however, with up to 35% of ALA being subjected to β-oxidation to produce energy and only around 5% of dietary ALA being converted into EPA (Burdge, 2004). Approximately 2-5% of this EPA is then further metabolised into DHA (Brenna, 2002). These conversion rates are dependent on the amount of n-6 circulating, however, as the same desaturase and elongase enzymes are utilised in the conversion of LA into PUFAs such as gamma-linoleic acid (GLA) (18:3n-6) and arachidonic acid (AA) (20:4n-6) (Lee *et al.*, 2016). The competing pathways involved in PUFA biosynthesis in humans can be seen in Figure 6. It is therefore advised that DHA and EPA are themselves directly obtained through the diet to make up for this low conversion rate, predominantly from marine dietary sources such as oily fish and algae (Bézard *et al*., 1994), and that the ratio of n-6 to n-3 PUFAs in the diet is also considered (Simopoulos, 2006).

PUFAs in the cell membrane

The fate of a number of these LC-PUFAs is their incorporation into cell membrane lipid molecules such as phospholipids. AA and DHA are the most commonly found PUFAs within these membrane lipid molecules throughout the entire body, but DHA is particularly plentiful in brain and retinal tissue, where it is around one hundred times more abundant than EPA (Arterburn *et al.,* 2006). The reason that DHA is so positively selected for plasma membrane integration is due to its uniquely high level of unsaturation causing a curved molecular structure. This curvature gives

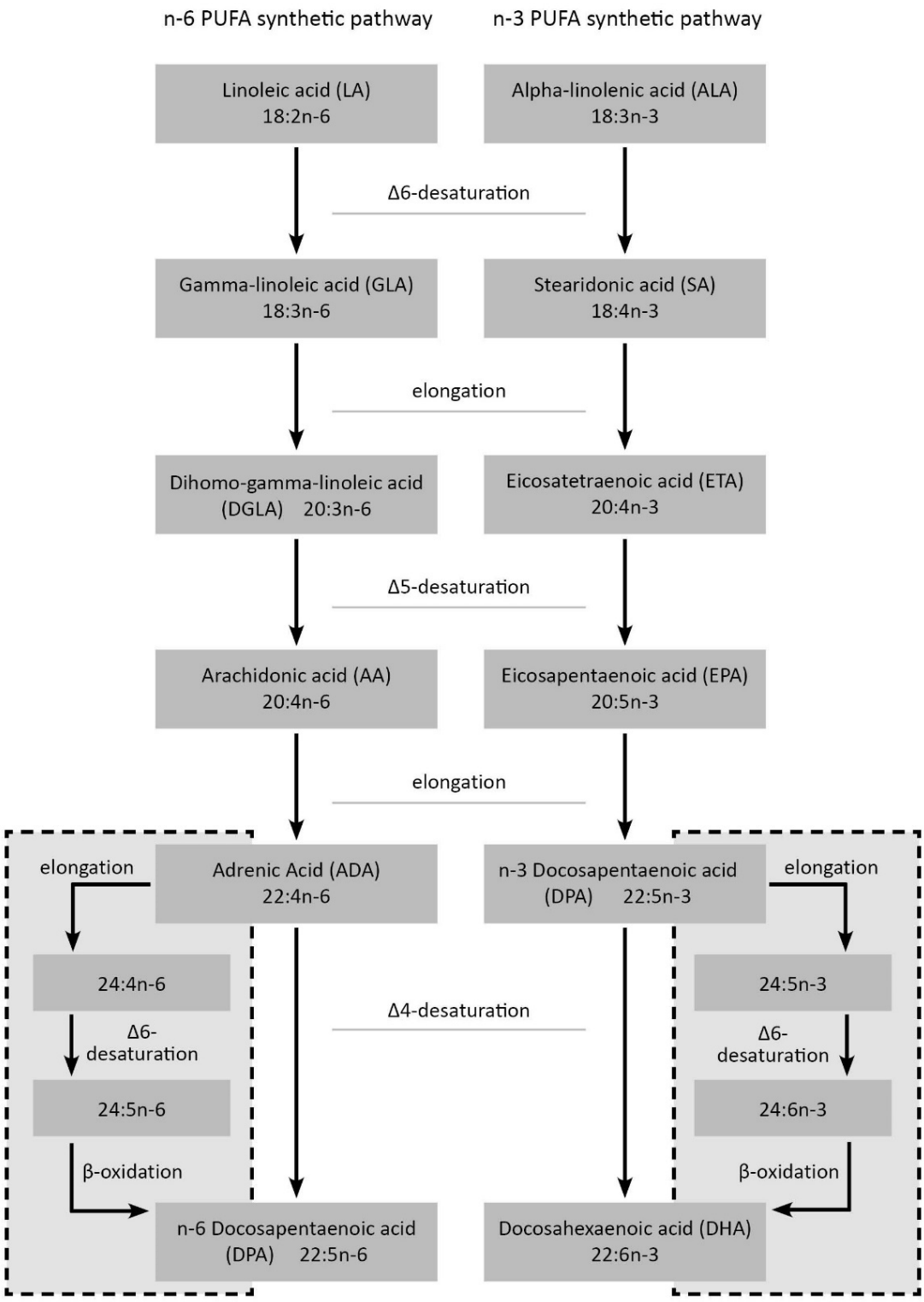

Figure 6. PUFA synthetic pathways in humans. Adapted from Lee *et al.,* 2016. The broken lines depict secondary pathways for the production of n-6 DPA and DHA.

valuable flexibility to the cell membrane in which it is incorporated, and this fluidity has substantial benefits for a wide range of cellular processes, particularly signal transduction (Hishikawa *et al.,* 2017). If n-3 PUFAs are lacking in the diet, and there are subsequently low levels of DHA available, more highly saturated fats would instead be assimilated into cell membranes, giving membranes a more rigid structure and negatively affecting their function and viability. Cell membrane PUFAs are also key mediators of membrane protein function and gene transcription (Calder, 2012).

PUFAs and inflammation

From their position in the cell membrane, DHA and the 20 carbon PUFAs AA, dihomo gamma linolenic acid (DGLA) and EPA are key in the cell's response to inflammation. As the cell detects a relevant stimulus, these PUFAs are liberated from the phospholipid membrane through the action of phospholipase A2 enzyme and can then be converted into different eicosanoid molecules via numerous different pathways, using lipoxygenase (LOX), cyclooxygenase (COX) and cytochrome P450 enzymes. There are more than 20 eicosanoid-synthesising pathways for AA alone, and the metabolism of DGLA and EPA follow similar pathways and compete for the same enzymes. The result is a diverse array of molecules involved in inflammatory and immune responses, such as prostaglandins, thromboxanes, leukotrienes, lipoxins, hydroxyeicosatetraenoic acids (HETEs), eoxins and resolvins (Harizi *et al,.* 2008; Weylandt *et al.,* 2012). The precise actions of each of these molecules is complex, but those derived from AA tend to have pro-inflammatory consequences, while those from DGLA or EPA are generally considered anti-inflammatory, with the capability to counteract the negative effects of the AA-derived eicosanoids (Calder, 2017).

PUFA intake

There has been a vast amount of research into the effects of dietary PUFAs on CVD and the consensus is that a sufficient intake and ratio of these EFAs is crucial in the fight against CVD (Allayee *et al.,* 2009; Simopoulos, 2006; Visioli & Poli, 2020). Despite the potential for n-6 PUFAs to create an inflammatory environment, they are still considered beneficial in many other ways, including helping to reduce LDL cholesterol levels, and can therefore contribute to a reduction of CVD risk (Maki *et al.,* 2018; Wang, 2018). It is recommended that between 6-11% of daily energy intake should be from PUFAs with between 2.5-9% coming from the n-6 family, and 0.5-2% coming from n-3s, and a combined total of EPA plus DHA of 250 mg/day (FAO/WHO, 2010). This gives an n-6:n-3 ratio of approximately 5:1, although a ratio as close to 1:1 as possible is ideal (Simopoulos, 2006). There is a concerning estimation that the average Western diet these days

Table 1. Known benefits of n-3 PUFAs to aspects of human health, besides CVD.

Known benefit	Reference/s
Depression and other psychiatric disorders n-3 PUFAs, but especially EPA, can improve symptoms of depression and other psychiatric disorders thanks to the ability to reduce inflammation, and in their role as an integral part of neuronal plasma membranes.	Grosso *et al.,* 2014 Liao *et al.,* 2019. Peet & Stokes, 2005
Eye health Retinal cells require DHA, of the n-3 PUFA family, as a major structural component, so diets lacking in DHA can lead to an increased risk of macular degeneration, a disease of the retina that can cause blindness.	San Giovanni & Chew, 2005 Merle *et al.,* 2014
ADHD Children with ADHD tend to have lower levels of DHA, EPA and total n-3 PUFAs in their blood, and treatment with EPA in particular can reduce cognitive symptoms.	Chang *et al.,* 2019
Chronic inflammation n-3 PUFAs decrease the body's production of certain inflammatory molecules through competitive inhibition and alteration of inflammatory gene expression.	Ishihara *et al.,* 2019 Calder, 2017
Autoimmune disease The anti-inflammatory action of n-3 PUFAs can reduce the risk of developing auto-immune disorders such as multiple sclerosis or latent autoimmune diabetes, and can also reduce symptoms of some chronic autoimmune conditions such as lupus.	Löfvenborg *et al.,* 2014 Hoare *et al.,* 2016 Duffy *et al.,* 2004 Berbert *et al.,* 2005
Age-related cognitive decline A diet high in n-3 PUFAs can reduce age-related cognitive decline due to the role of these fatty acids in neuronal membrane integrity and fluidity, and inflammation reduction.	Solfrizzi *et al.,* 2006 Wood *et al.,* 2022
Cancer n-3s can affect tumour development through the capacity to reduce inflammation, and by interfering with eicosanoid production through competitive inhibition. Some eicosanoids are thought to encourage tumour growth.	Gomes *et al.,* 2018
Asthma A correlation between the amount of n-3 PUFAs consumed and the incidence of asthma seen in young adults has been observed. This is most likely thanks to the anti-inflammatory properties of n-3 PUFAs.	Li *et al.,* 2013 Ekström *et al.,* 2022
Fatty liver disease Numerous studies show that a high dietary n-3 PUFA intake can reduce lipid build-up in the liver, preventing fatty liver disease, and reducing the risk of developing cirrhosis and liver cancer.	Jump *et al.,* 2015 Scorletti & Byrne, 2018
Bones and joints n-3 PUFAs are involved in the process of calcium absorption and deposition in the body, and so increasing intake can enhance bone density. Rheumatoid arthritis sufferers can also benefit from the anti-inflammatory properties of n-3s.	Kruger *et al.,* 1998 Veselinovic *et al.,* 2017
Menstrual pain Various types of pain can be successfully treated with n-3 PUFAs, but the biggest effect is seen with menstrual pain, aka dysmenorrhea.	Prego-Dominguez *et al.,* 2016
Sleep Studies have demonstrated a relationship between n-3 PUFA intake and sleep/wake activity. It is thought that this could be in part due to interactions between n-3 PUFA molecules and serotonin receptors.	Decoeur *et al.,* 2020 Del Brutto *et al.,* 2016,
Skin n-3 PUFAs play important roles in cell membrane structure and inflammation. Further to this, LA is the most abundant FA found in the skin's epidermis, so n-6 PUFAs are also important for healthy skin.	McCusker & Grant-Kels, 2010.

Abbreviations: ADHD = Attention deficit hyperactivity disorder; CVD = Cardiovascular disease; DHA = Docosahexaenoic acid; EPA = Eicosapentaenoic acid; LA = Linoleic acid; FA = Fatty acid; PUFA = Polyunsaturated fatty acid.

sees ratios closer to 15:1-17:1, and one review of a number of studies focusing on European populations determined that about 50% of people are falling well short of recommended daily PUFA intakes (Eilander *et al.,* 2015). It is therefore vital that public health messages relating to improvements in PUFA intake are widely promoted and heeded. As well as being advantageous in the fight against CVD, numerous clinical studies and reviews have concluded that n-3 PUFAs are beneficial for a whole range of human health conditions, with effects such as improvement in sleep, reduction in pain and relief of symptoms of depression. A number of these are detailed in Table 1.

Of particular interest for this study is the positive effect that n-3 PUFAs appear to have on the health of the heart, including the incidence of arrhythmias, as ventricular arrhythmias account for approximately 80% of sudden cardiac deaths, which themselves make up around 50% of total deaths resulting from CVD (Mehra, 2007). It is thought that these FAs interact in an advantageous manner with the sodium and potassium ion channel membrane proteins that are key in the efficient propagation of the heart's action potentials during the cardiac cycle (Moreno *et al.,* 2012).

The cardiac cycle and cardiovascular conduction system

A full cardiac cycle covers the time from the start of one heartbeat to the beginning of the next, which is approximately 0.8 seconds where the heart rate is around 75 beats per minute. The cycle is characterised by periods of synchronised relaxation and contraction of the atrial and ventricular muscles, beginning with the relaxation of both the left and right ventricles, otherwise known as ventricular diastole. The atria are already in a diastolic state at the beginning of the cycle, and so during this period the ventricles are passively filling with blood via the pulmonary vein and the vena cava. The atria then contract, otherwise known as systole, which forces extra blood through the tricuspid and mitral valves, into both ventricles, before the final stage, which is ventricular contraction/systole. As the ventricles contract, the tricuspid and mitral valves are forced shut so pressure within the chambers rises, ejecting blood from them. From the right ventricle, the blood flows via the pulmonary artery to the lungs, to be oxygenated, before returning to the heart via the pulmonary veins and into the left atrium. From the left ventricle, the blood is ejected under greater pressure, via the aorta, through the systemic circulation, to the cells of the body (Figure 7) (Sherwood, 2013).

The continual cycle of co-ordinated contraction and relaxation of heart muscle cells (myocytes) is due to rhythmical electrical activity, known as the pacemaker-conduction system. This electrical

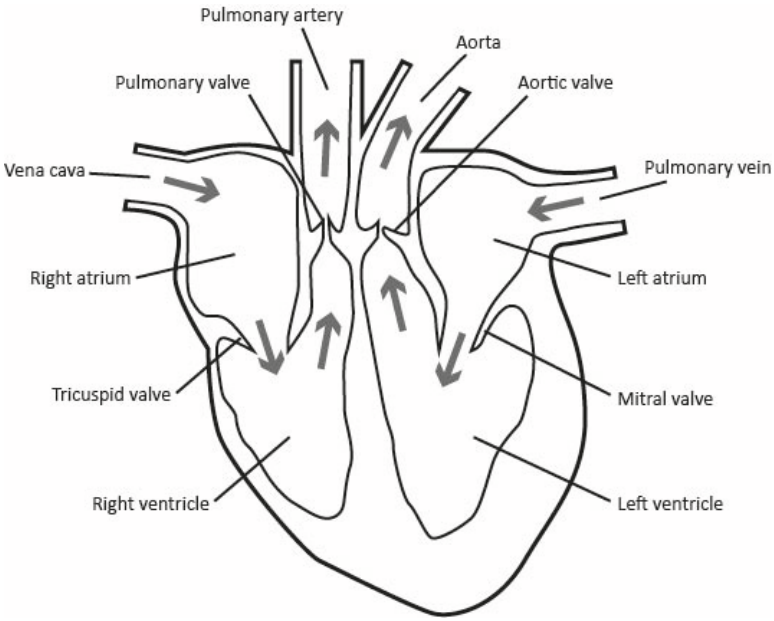

Figure 7. The structure of the heart and the flow of blood through it. Redrawn, adapted from Sherwood (2013).

activity originates in specialised self-excitable muscle cells, concentrated in nodes in the wall of the heart. Beginning from the sinoatrial node (SAN), spontaneously generated action potentials (APs) travel quickly from cell to cell throughout the myocardium, as ions move through special ion channels in gap junctions between cells (Kennedy *et al.,* 2016). The flow of these ions alters the voltage across the membrane of the cell. The membrane potential of myocardial cells is around -90mV when at rest, brought about by pumps that maintain a specific gradient of ions inside and outside of the cell, and so the cell is polarised. At rest, Ca^{2+} and Na^{+} ions predominate in the extracellular fluid, and there are more K^{+} ions inside the cell. An action potential in an adjacent cell causes the polarity of the cell membrane to decrease. When this voltage decreases to a particular level (-70mV), a threshold is reached, opening fast Na^{+} channels, allowing Na^{+} to move into the cell, causing a rapid rise in voltage. This phase is known as the depolarisation phase. When the polarity of the cell membrane reaches -40mV more ions channels open, resulting in a slow influx of Ca^{2+} ions (Klabunde, 2017; Levick, 2012).

The AP reaches a peak level, at which point the Na^{+} channels quickly close, and K^{+} channels open, beginning a phase called early repolarisation, as there is a small decrease in voltage across the cell membrane. Ca^{2+} ions are the ones which set off the contractile units of the cell, called sarcomeres, and the cell itself holds a store of Ca^{2+} ions within its sarcoplasmic reticulum (SR). These stores are released into the myocyte cytoplasm at this point, bringing about a plateau in membrane voltage and activating the sliding filament mechanism, which is how the muscle

contracts. After contraction, Ca^{2+} channels then close and Ca^{2+} ions are actively transported from the cytoplasm back into the SR and the extra cellular fluid and, with the help of the Na^{+}/K^{+} pump, the membrane potential returns to the resting value of -90mV in a phase called repolarisation (Klabunde, 2017; Levick, 2012).

APs propagate through the conduction system in this way, from the SAN, initially to the atria, and then to the atrioventricular node (AVN) which slows the signal slightly. From here, APs travel down the bundle of His, to the Purkinje fibres of the left and right bundles around the heart's apex (Figure 8.) (Kennedy *et al.,* 2016). This precise flow of electrical activity, mediated by the actions of membrane PUFAs, facilitates the co-ordinated contraction and relaxation of the atrial and ventricular muscle previously described, which allows efficient pumping of blood through the systemic circulation.

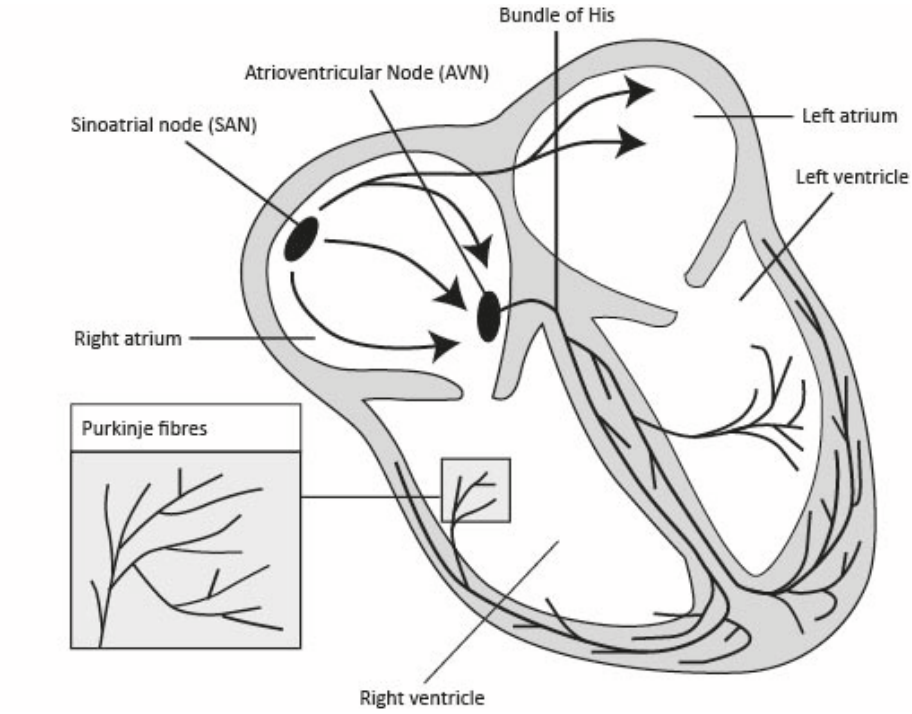

Figure 8. The path of AP propagation through the heart's conduction system. Redrawn, adapted from Levick, 2012.

The electrocardiogram

An electrocardiogram (ECG) is a clinical test of the cardiac conduction system, and entails placing electrodes at specific points on the body so that the heart's changing electrical activity can be detected and graphically represented. ECG read-outs are printed on to special gridded paper

where 1mm along the x-axis equates to 0.04 seconds, and 1mm on the y-axis represents 0.1mV. It is a particularly useful tool for picking up abnormalities in the cardiac conduction system, which can signify disease e.g. arrhythmia and myocardial ischemia. Figure 9 is a graphical representation of the electrical activity over time during a single heartbeat, as it might appear on an ECG, plus the naming of the standard features.

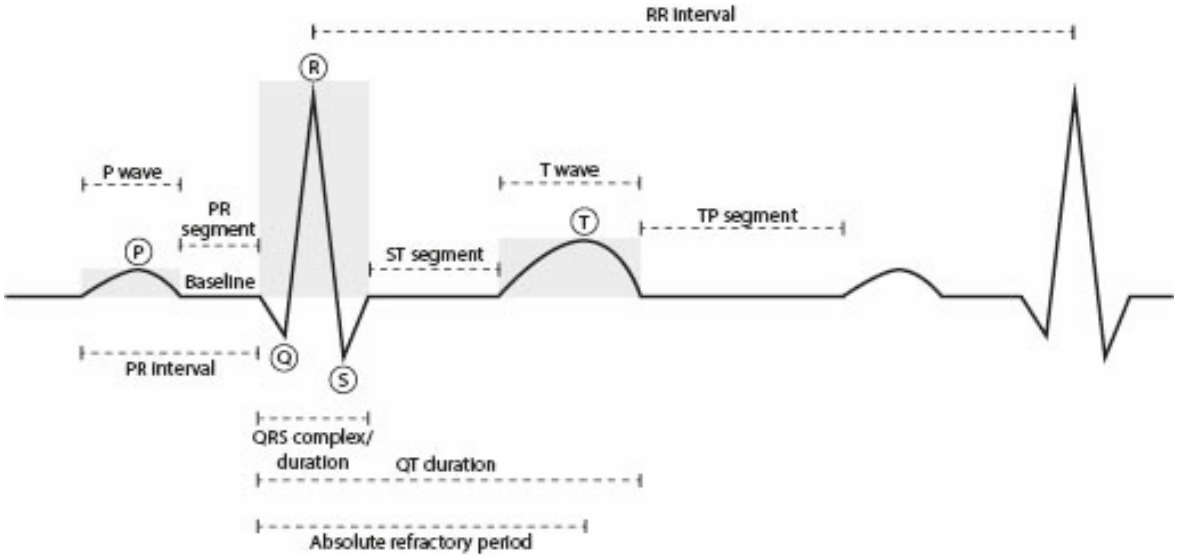

Figure 9. A graphical representation of the changes in electrical activity during a heartbeat. Grey zones illustrate the wave amplitudes, measured from the baseline. Adapted from Levick, 2012.

The QRS complex on this ECG read-out is the electrical activity that relates specifically to the contraction of the ventricles – ventricular depolarisation – and is the parameter of greatest interest for this study. Measuring the duration of the complex (QRS duration), the height of the wave from the baseline (R wave amplitude), and the area under the 'curve' (AUC of the QRS) provides a quantifiable representation of ventricular function at this point in the cardiac conduction cycle. Enlargement of ventricular muscle (hypertrophy) is common in conditions such as hypertension and atherosclerosis and the effect of this is a delay in the depolarisation process, and/or an increase in electrical activity, seen as a lengthening of the QRS duration, an increase in R wave amplitude and/or an increase in the AUC (Kahan & Bergfeldt, 2005). Table 2 provides additional details on all phases of the cardiac cycle, and their clinical significance.

Table 2. The different phases of the ECG and what they signify.

Phase of the ECG	Relates to	Signifies
P wave duration and P wave amplitude	Atrial depolarisation	A wide or a high P wave can be indicative of enlarged atria. Normal duration should be <0.12 seconds, and amplitude should be <2.5mm/0.25mV.
PR interval	The conduction of the AP from the atria to the ventricles, via the AVN.	A prolonged PR interval can indicate delayed signal conduction due to some sort of block. A short interval might indicate that the AP is bypassing the AVN. A normal PR interval should be between 0.12-0.2seconds.
PR segment	The short period of time between atrial and ventricular depolarisation	This flat segment is used as the baseline to measure all other segments against. PR segment anomalies are uncommon.
AUC of QRS and QRS duration	Ventricular depolarisation	Large area or long duration means that depolarisation is slow, which could indicate a dysfunction in the heart's conduction system. Normal duration should be <0.12 seconds (120 milliseconds).
R wave amplitude	The voltage change during ventricular depolarisation	A high wave could indicate ventricular hypertrophy as electrical activity detected relates to the mass of the muscle. Normal amplitude should be ≤20mm/2mV.
QT duration	Ventricular depolarisation and repolarisation.	A reduced or prolonged QT duration can imply certain diseases or imbalances in electrolytes. It can also be a result of a number of medications. Normal QT duration in women should be between 0.44-0.46 seconds.
ARP	The time taken from the beginning of ventricular depolarisation almost to the end of repolarisation, at the peak of the T wave.	It is the time period during which a new AP cannot be generated. A prolonged ARP could indicate a dysfunction in the Na^+ ion channels. Should be approximately 250 milliseconds.
ST segment	The period of time between ventricular depolarisation and repolarisation.	Position above or below the baseline is more significant than duration.
T wave duration and amplitude	Ventricular repolarisation	T wave amplitude can be highly variable, so variations are not uncommon. However, a high amplitude could imply an electrolyte imbalance.
TP segment	The time between ventricular repolarisation and the beginning of atrial depolarisation	As it represents a short period of electrical inactivity, it is expected to be at baseline level. Duration shortens as heart rate increases.
RR interval	From the apex of one R wave, to the apex of the next, this relates to heart rate	The smaller the interval, the higher the heart rate. Normal heart rate is between 60-100 beats per minute.

Table compiled using information from Levick (2012) and Jarvis (2021). Grey rows correspond to those phases that are the most significant, in a clinical setting, in identifying cardiovascular problems, and therefore the measurements made on the ECG read-outs in this study.
Abbreviations: AP = Action potential; ARP = Absolute refractory period; AUC = Area under the curve; AVN = Atrioventricular node; SAN = Sinoatrial node.

Aims and objectives

The aim of this observational study is to investigate whether anthropometric biomarkers, nutrient intake and blood fatty acid composition are associated with the electrical activity of the heart in a sample population of healthy women, to corroborate known links between fatty acids and aspects of cardiac function.

Using anthropometric and cardiovascular data, as well as ECGs, capillary blood samples and 4-day food diaries from recruited participants, enough data can be garnered on dietary intakes and blood fatty acid levels to test correlations between these and ECG measurements, plus other markers of cardiovascular health, by way of statistical analysis. A particular focus on any relationships between n-3 PUFAs and ventricular depolarisation (AUC of the QRS, QRS duration and R wave amplitude), can then be used to support the growing body of evidence that n-3 PUFAs interact with ion channels in plasma membranes and have anti-arrhythmic properties.

METHODS

Ethical approval

Participant information sheets, questionnaires, food diaries and informed consent forms were designed and sent to the University of Worcester Ethics Committee, along with risk assessments, for ethical approval, which was subsequently granted (Appendix A).

Participant recruitment

24 pre-menopausal, non-smoking females between the ages of 25 and 40 were recruited. Age is a known risk factor for CVD (Rodgers, *et al.,* 2019), as is the menopause, due to the many physiological and hormonal changes that occur during this transitional period (El Khoudary & Thurston, 2018). Smoking has been proved to increase the risk of 80% of all subtypes of CVD (Banks *et al.,* 2019), so the potential effects of these important confounding variables were either eliminated or reduced.

Those with liver, kidney, lung or heart disease, type 2 diabetes or psychiatric disorders were excluded so as to ensure the sample was taken from a healthy population with an average CVD risk for age. Those with any condition, or taking any medication, known to affect heart rhythm were excluded so that ECG results were not affected.

Participant information sheets were provided, and signed consent forms were obtained. All data collected was anonymised from the outset, to maintain confidentiality.

Laboratory testing

Each participant was encouraged to eat a normal breakfast on the morning that they each attended the laboratory for the tests, and all were seen in the morning, before lunch, to ensure consistency. All tests were carried out according to the risk assessments seen in Appendix A.

Anthropometrics

Questionnaires were filled in, and then height measurements, in centimetres, were taking using a Leicester height measure, and a Seca weighing scale was used to determine weight in kilograms. Bioelectrical impedance monitoring, using an Omron Body Fat Monitor (model BF306), was carried out to record BMI and percentage body fat. The World Health Organisation's anthropometrical guidelines (WHO, 1995) were used to ensure consistency when taking waist and hip measurements, using a tape measure. Waist to hip ratio was calculated from these measurements.

Cardiovascular tests

Participants were seated and a fingertip pulse oximeter was used to quantify blood oxygen levels and heart rate, while blood pressure was taken using an Omron automatic blood pressure monitor. Mean arterial pressure (MAP) was calculated using the formula MAP = DBP + 1/3(SBP-DBP), where SBP is systolic blood pressure and DBP is diastolic blood pressure.

Electrocardiography

ECGs were performed on participants while in a supine position, using a Seca CT8000i with the four limb leads only, placed on both wrists and ankles. 12-lead ECGs are standard in clinical settings for diagnostic purposes as they can pinpoint specific problem areas in patients with known cardiovascular issues (Alinier *et al.,* 2006). For the level of this investigation, where the requirement was a simple representation of the electrical activity on a single plane, and because all of the participants were considered healthy, a 4-lead ECG was deemed sufficient. At least twenty full cardiac cycles were recorded for each participant.

Blood sample collection

Participants were asked to wash their hands and a finger prick procedure, using sterile lancets, was followed in order to obtain two samples of capillary blood. One was taken into a clean capillary tube, which was sealed and spun in a Pico 17 centrifuge for five minutes at 8000rpm. Once spun, the haematocrit level was calculated by measuring the volume of red blood cells as a percentage of the whole blood volume. The second blood sample was taken on to a small circle of sterile Whatman filter paper which was then wrapped in clean foil and placed overnight in a dessicator to dry out, before being moved to a -80°C freezer for storage before methylation at a later date.

Fatty acid methylation

FAs present in blood samples had to be converted into fatty acid methyl esters (FAMEs) before analysis by gas chromatography – flame ionisation detection (GC-FID) could take place. The processes involved in fatty acid methylation, FAME extraction, GC-FID separation and analysis have been described previously by our group (Joyce, 2022; Boldarine *et al.,* 2021; Hirata *et al.,* 2019; Bueno *et al.*, 2015). The following method descriptions have been reproduced with permission from E.C Joyce (2022).

Glassware:

Glassware used for the methylation process was cleaned using a bath of 10% nitric acid. After soaking for a minimum of 2 hours, glassware was washed twice at 70°C, the first time using phosphate-free detergent, and the second time using no detergent. It was then rinsed thoroughly in de-ionised water and dried in a drying cabinet.

Methylation process:

Methylating reagent was freshly prepared by slowly adding 15ml of acetyl chloride to 100ml dry methanol in a 500ml conical flask. This was done in a fume cupboard and over ice to reduce the risk of the mixture boiling.

Filter paper blood samples that had been stored at -80°C, were trimmed so that only blood-saturated paper was used, and these samples were each placed into separate methylating tubes and appropriately labelled. Two control tubes were also prepared: one which contained a small square of clean filter paper, and one which contained no paper. 4ml of methylating reagent was added to each tube and they were flushed with oxygen-free N^2 (OFN). Caps were secured on tubes, the level of liquid on each tube was marked, and samples were placed in an oven at 70°C for three hours. After one hour and two hours the samples were vortexed and checked for evaporation of methylating reagent. Where evaporation had occurred, levels were topped up to the marked line with methanol before being returned to the oven. Once removed from the oven and cooled, 4ml 5% saline solution and 2ml petroleum spirit + butylated hydroxy toluene (BHT) was added, samples were shaken, but not vortexed, and the upper petrol layer was removed to a test tube that contained 2ml 2% potassium bicarbonate. An additional 1ml of petroleum spirit was added and samples were shaken, and the upper layer was again removed to the tube containing potassium bicarbonate. This addition and removal of 1ml petroleum spirit was repeated once more, so that in total a 4ml extract was collected from each sample.

The petrol extracts were placed in a centrifuge at 1500rpm for five minutes and the upper petrol layer from each sample was transferred to a separate test tube that contained 100-200g of dried, granular sodium sulphate. Samples were vortexed and each solution was transferred to a 3ml vial, being careful to avoid transference of any sodium sulphate granules. Samples were flushed with OFN at 37°C to evaporate the petrol. Evaporated samples were resuspended in 1ml heptane + BHT, flushed again with OFN, and stored at -20 °C.

Gas chromatography – flame ionisation detection (GC-FID)

To identify FAs in samples, retention times as compared to known standards must be determined. A Shimadzu GC – 2010 Plus machine and a Shimadzu – AOC-20S autosampler (Shimadzu, Kyoto, Japan) were used in the separation of FAMEs for analysis. A Peak Scientific zero air precision compressor (Peak Scientific Instruments, Scotland, UK) and SGE Analytical Science™ BPX70 GC Capillary Column (120m x 0.25 mm x 0.25 µm – Code: 054624; Milton Keynes, United Kingdom) were fitted to this, and the following flow rates were set to the system: Nitrogen (carrier gas) – 30ml/minute; hydrogen – 40ml/minute; air flow – 400ml/minute; septum purge flow – 3ml/minute. Temperatures of the injection port and FID detector were set at 230 °C and 260 °C, respectively. Acetone and heptane were used to rinse the needle before and after each injection, with additional heptane being injected after every 5 samples to help identify any ghost peaks.

FAME samples and blanks were retrieved from the freezer, resuspended in 250µl heptane + BHT and transferred to gas chromatography vials. The volume of each sample injected was set to 1µl, with a split ratio of 1:100, and the following method was run for each: Hold at a start temperate of 130°C for 1 minute. Increase temperature by 5.5°C every minute until 200°C, then hold for 3.5 minutes. Increase by 12°C per minute until 250°C, then hold for 3 minutes. Increase by 10°C per minute until 260°C, then hold for 2 minutes. The system was equilibrised for 2 minutes between each sample injection.

Outputs were blanked against controls and then peak areas were analysed using Shimadzu LabSolutions software (Shimadzu, Kyoto, Japan), with a full list of FAME standards used in this process seen in Appendix B. Table 3 shows a list of the FAs quantified using GC-FID.

Dietary analysis

Food diary templates (Appendix B) were provided to participants, who recorded everything they ate and drank over a consecutive four-day period, covering two weekend days and two week days. Gersovitz *et al*. (1978) consider four days to be sufficient for this method of data collection as a participant's motivation to continue recording information tends to wane if the time period is made any longer. Food diaries were collected and analysed using Nutritics (2022), the outputs giving the amounts of each nutritional component reportedly consumed by each participant per day. The four daily totals were then averaged for each participant, to give one final figure for each nutrient.

Table 3. Fatty acid (FA) families, with the common and numeric names of each FA quantified using gas chromatography – flame ionisation detection (GC – FID).

FA family/sub-family	Common name of FAs in family	Numeric name
Saturated fatty acids (SFAs)		
	Myristic acid	C14:0
	Palmitic acid	C16:0
	Stearic acid	C18:0
	Arachidic acid	C20:0
	Behenic acid	C22:0
	Lignoceric acid	C24:0
Monounsaturated fatty acids (MUFAs)		
n-7	Palmitoleic acid	C16:1n-7
	Vaccenic acid (VA)	C18:1n-7
n-9	Oleic acid	C18:1n-9
	Eicosenoic acid	C20:1n-9
	Erucic acid	C22:1n-9
	Nervonic acid (NA)	C24:1n-9
Polyunsaturated fatty acids (PUFAs)		
n-6		
	Linoleic acid (LA)	C18:2n-6
	Gamma-linolenic acid (GLA)	C18:3n-6
	Eicosadienoic acid (EDA)	C20:2n-6
	Dihomo gamma linolenic acid (DGLA)	C20:3n-6
	Arachidonic acid (AA)	C20:4n-6
n-3		
	Alpha linolenic acid (ALA)	C18:3n-3
	Eicosatrienoic acid	C20:3n-3
	Eicosapentaenoic acid (EPA)	C20:5n-3
	Docosapentaenoic acid (DPA)	C22:5n-3
	Docosahexaenoic acid (DHA)	C22:6n-3
Di-methyl acetals (DMAs)		
	Hexadecanal dimethyl acetal	DMA16:0
	Octadecanal dimethyl acetal	DMA18:0

ECG analysis

ECG print-outs, representing at least 20 cardiac cycles for each participant, were scanned to create digital files and, using Adobe Acrobat Pro software (version 2022.003.20282), various measurements from those cardiac cycles were taken in millimetres (mm). The parameters measured were: AUC of the QRS complex, PR interval, QRS duration, P wave duration, QT duration, absolute refractory period (ARP), R wave amplitude, P wave amplitude (see Figure 9). The AUC of the QRS was measured using the lowest part of the Q wave, and the lowest part of the S wave, as shown in Figure 10, and recorded in mm^2. The measurements taken along the x axis were converted from mm to milliseconds (ms) as 1mm on an ECG strip represents 0.04 seconds. Measurements taken on the y axis (amplitudes) were converted from mm to millivolts (mV) as 1mm is equal to 0.1mV. Values were then averaged, to give one reading for each parameter, per participant.

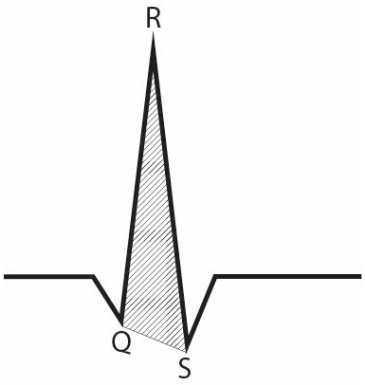

Figure 10. Diagram showing how the AUC of the QRS was measured.

Statistical analysis

JASP software (JASP Team, 2022) was used to analyse data. Shapiro-Wilk tests for bivariate normality were performed on pairs of variables. Associations between variables were tested using the relevant Pearson's or Spearman's rho test, according to the normality of the distributions. The significance threshold was set at $p < 0.05$.

RESULTS

Raw data can be found in Appendix C.

Anthropometric data

Means and standard deviations for anthropometric data collected can be seen in Table 4. The participants were all female and between the ages of 25 and 40, with most in the latter half of that age range, giving a mean age of 36. Mean BMI was 25.79, with at least one participant in all of the four categories: underweight, healthy weight, overweight and obese. Waist to hip ratio was calculated by dividing the measurement for waist circumference by that of the hip circumference.

Table 4. Means and standard deviations for anthropometric and cardiovascular data

Variable measured	Mean	SD
Age	36.09	± 4.16
Height (cm)	166.73	± 7.03
Weight (kg)	71.96	± 14.72
Waist circumference (cm)	80.04	± 8.55
Hip circumference (cm)	104.28	± 9.55
Waist:hip ratio	0.77	± 0.04
Body fat %	36.29	± 5.11
BMI	25.79	± 5.25
Haematocrit (%)	39.52	± 2.35
Heart rate (bpm)	67.91	± 8.15
Pulse ox (%)	98	± 0.60
SBP (mmHg)	117.52	± 10.68
DBP (mmHg)	76.96	± 8.55
MAP (mmHg)	90.46	± 8.46

Abbreviations: BMI = Body mass index; bpm = beats per minute; DBP = Diastolic blood pressure; MAP = Mean arterial pressure; SBP = Systolic blood pressure; SD = Standard deviation

Nutritics – Dietary analysis

Means and standard deviations for intakes of nutritional components from Nutritics outputs can be seen in Table 5. These figures were used to calculate the mean daily intakes of the main macronutrients as a percentage of total energy, and these can be seen in Table 6, along with what is recommended by the Department of Health (DoH, 1991).

Average calorific intake was approximately 1500kcal, but with a standard deviation of ±409, this varied greatly from one participant to another. Carbohydrate was the most abundant macronutrient consumed, however, the mean intake as a percentage of the total energy intake was slightly less than is recommended. SFA intake as a percentage of total energy was higher than

recommended, but the mean daily intake by weight was within recommended limits. The intakes of both MUFAs and PUFAs as a proportion of energy intake were lower than ideal. TFA intake was very low. The n-6:n-3 ratio was calculated by dividing the total for n-6 by the total for n-3, and was calculated as 5.55.

Table 5. Means and standard deviations for daily intakes over a four-day period, by dietary analysis.

Nutritional component	Mean	SD
Energy (kcal)	1578	± 409
Carbohydrates (g)	164.35	± 65.18
Protein (g)	70.37	± 15.77
Total fat (g)	64.54	± 19.49
SFA (g)	23.41	± 8.27
MUFA (g)	15.61	± 7.13
PUFA (g)	7.15	± 3.54
N-6 total (g)	3.38	± 2.25
N-3 total (g)	0.906	± 0.823
n-6:n-3 ratio	5.55	± 4.57
TFA (g)	0.443	± 0.274
Cholesterol (mg)	199.15	± 125.48

Abbreviations: kcal = kilocalories; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; SD = Standard deviation; SFA = Saturated fatty acid, TFA = Trans fatty acid.

Table 6. Mean macronutrient intakes as a percentage of total energy consumption, versus recommended proportions

Nutrient	Energy (kcal) calculated from mean intakes (g) and kcal/g of macronutrient*	Mean intakes as a % of total energy**	Recommended % of daily energy intake***
Carbohydrate	657.42	41.67	45-65
Protein	281.48	17.84	15
Total fat	580.86	36.81	33-35
SFA	210.69	13.35	10
MUFA	140.49	8.90	12
PUFA	64.35	4.08	6
TFA	3.96	0.251	2

*Based on the assumption that carbohydrates and protein provide 4kcal/g, and fats provide 9kcal/g (SACN, 2011). **Where mean total energy intake was 1578 kcal. ***As recommended by the Department of Health (DoH, 1991). Abbreviations: kcal = kilocalorie; MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; SFA = Saturated fatty acid; TFA = Trans fatty acid.

ECG analysis

One participant was eliminated due to an anomaly in their ECG read-out which warranted medical investigation, leaving 23 participants in total. Measurements for each parameter from at least 20 cardiac cycles were averaged to give one reading, and means and standard deviations for these measurements can be seen in Table 7, along with a comparison to normal limits. AUC of the QRS has no defined normal range, and ARP was the only mean that was elevated as compared to what is considered normal.

Table 7. Means and standard deviations for the relevant sections of the cardiac conduction system.

Section of the ECG	Mean	SD	Normal limits
AUC of QRS complex (mm^2)	7.74	± 2.23	-
QRS duration (ms)	81.25	± 9.13	70-104[a]
R wave amplitude (mV)	1.08	± 0.31	<2mV[c]
PR interval (ms)	149.13	± 21.06	118-212[a]
P wave duration (ms)	93.30	± 12.80	<110[b]
P wave amplitude (mV)	0.13	± 0.03	<0.25mV[c]
QT interval (ms)	393.06	± 20.51	388-450[a]
ARP (ms)	310.21	± 17.06	Approx. 250

[a] Rautaharju *et al*., 2013; [b] Vepsäläinen *et al.,* 2014; [c] Meek & Morris, 2002. Abbreviations: ARP = Absolute refractory period; AUC = Area under the curve; SD = Standard deviation.

Fatty acid analysis – GC-FID

Means and standard deviations for data collected can be seen in Table 8. The most abundant FAs identified in blood samples were the SFA C16:0 (stearic acid) – 23%, the MUFA C18:1n-9 (oleic acid) – 20%, and the n-6 PUFA C18:2n-6 (linoleic acid) – 23%. The least abundant were C20:2n-6 (eicosadienoic acid - EDA) - 0.098% and C22:1n-9 (erucic acid) – 0.06%. The ratio of n-6 PUFAs to n-3 was 6.75, and there was a greater proportion of total PUFAs in the blood (38%) than either MUFAs (25%) or SFAs (35%).

Table 8. Means and standard deviations for the percentages of total FA detected in participants' capillary blood samples using GC-FID.

Fatty acid (% of total FAs)	Mean	SD
C14:0	0.829	± 0.480
C16:0	23.332	± 2.119
C18:0	8.786	± 2.134
C20:0	0.115	± 0.043
C22:0	0.304	± 0.141
C24:0	1.943	± 0.440
SFA total %	35.308	± 2.829
C16:1n-7	1.204	± 0.781
C18:1n-9	20.483	± 2.374
C18:1n-7	1.556	± 0.365
C20:1n-9	0.397	± 0.143
C22:1n-9	0.066	± 0.030
C24:1n-9	1.487	± 0.350
MUFA total %	25.193	± 3.000
C18:2n-6	22.692	± 3.556
C18:3n-6	0.132	± 0.064
C20:2n-6	0.098	± 0.018
C20:3n-6	1.247	± 0.283
C20:4n-6	8.769	± 1.501
n-6 total %	32.939	± 3.685
C18:3n-3	0.289	± 0.135
C20:3n-3	0.260	± 0.146
C20:5n-3	0.528	± 0.238
C22:5n-3	0.968	± 0.198
C22:6n-3	2.909	± 0.642
n-3 total %	4.953	± 0.788
PUFA total %	37.89	± 4.189
n-6:n:3 ratio	6.752	± 0.924
DMA 16:0	0.295	± 0.060
DMA 18:0 A	0.371	± 0.100
DMA 18:0 B	0.941	± 0.217

See Table 3 for the common names of the FAs listed. Abbreviations: DMA = Di-methyl acetal; FA = Fatty acid; GC-FID = Gas chromatography - flame ionisation detection; MUFA = Monounsaturated fatty acid; SD: Standard deviation; SFA = Saturated fatty acid.

Statistical analysis

All significant correlations that resulted from statistical analysis were collated and can be found in Appendix D. Those relevant to the objective of the study are flagged in this chapter.

ECG parameters

Table 9 details the statistically significant correlations involving the 3 ECG phases that directly represent ventricular depolarisation i.e. AUC of the QRS, QRS duration and R wave amplitude. Where a significant relationship was found, the relevant correlation co-efficient, dependant on whether a Pearson's or a Spearman's rho correlation was carried out, and p value, are stated.

Table 9. Statistically significant correlations with the ECG phases that represent ventricular depolarisation

Variable	ECG phase		
	AUC of QRS	QRS duration	R wave amplitude
Weight (kg)	-	r = 0.427, p = 0.047	-
BMI	-	rho = 0.418, p = 0.047	-
Waist circumference (cm)	-	r = 0.428, p =0.042	-
Hip circumference (cm)	-	r = 0.492, p = 0.017	-
Energy (kcal)*	r = 0.574, p = 0.04	-	r = 0.593, p = 0.003
Carbohydrates (g)*	r = 0.452, p = 0.03	-	r = 0.497, p = 0.016
Total fat (g)*	r = 0.661, p = <0.001	-	r = 0.573, p = 0.04
SFA (g)*	r = 0.549, p = 0.007	-	r = 0.480, p = 0.021
% of energy as protein*	r = -0.492, p = 0.017	-	r = -0.468, p = 0.024
% of energy as fat*	-	rho = 0.468, p = 0.024	-
% of energy as SFA*	-	rho = 0.446, p = 0.033	-
% C18:1n7**	r = -0.460, p = 0.022	-	r = -0.440, p = 0.036
% C22:6n3**	r = -0.463, p = 0.026	-	-
n6:n3 ratio**	r = 0.576, p = 0.004	-	rho = 0.423, p = 0.045

*Value ascertained from dietary analysis
**Value ascertained from GC-FID
r = Pearson's correlation coefficient; rho = Spearman's rank correlation coefficient
Abbreviations: AUC = Area under the curve; BMI = Body mass index; ECG = Electrocardiogram; SFA = Saturated fatty acid

AUC of the QRS and R wave amplitude shared 7 of the same significant correlations. There were positive relationships between each of these parameters and the dietary intakes of total energy, carbohydrates, total fat and SFA. Also established were negative associations between the same 2 parameters and the proportion of FAs in the blood made up from C18:1n7 (vaccenic acid - VA), and the percentage of energy taken in as protein. The ratio of n-6:n-3 PUFAs was also seen to correlate positively with both AUC and R wave amplitude. In addition, a significant inverse association was seen between AUC of the QRS and the proportion of C22:6n3 (DHA) identified in the blood. The duration of the QRS correlated significantly and positively with 4 markers of obesity (weight, BMI, waist circumference and hip circumference), as well as proportion of energy taken in as both fat and SFA.

Table 10 details the statistically significant correlations involving the 5 remaining ECG phases. Where there was a significant relationship found, the correlation co-efficient and p value are stated.

Table 10. Statistically significant correlations with the remaining phases of the ECG.

Variable	Phases related to P wave/atrial depolarisation			Phases incorporating QRS and T wave	
	PR interval (ms)	P wave duration (ms)	P wave amplitude (mV)	QT duration (ms)	ARP (mV)
Weight (kg)	r = 0.475, p = 0.026	r = 0.43, p = 0.046	-	-	-
Hip circ. (cm)	r = 0.437, p = 0.037	rho = 0.467, p = 0.025	-	-	-
Energy (kcal)*	-	-	-	r = -0.549, p = 0.007	rho = -0.575, p = 0.004
Carbohydrates (g)*	-	-	-	r = -0.489, p = 0.018	r = -0.485, p = 0.019
Protein (g)*	-	-	-	rho = -0.709, p = <0.001	rho = -0.695, p = <0.001
Total fat (g)*	-	-	-	r = -0.426, p = 0.043	-
SFA (g)*	-	-	-	r = -0.425 p = 0.043	r = -0.457, p = 0.028
MUFA (g)*	-	-	-	r = -0.422 p = 0.045	-
TFA (g)*	-	-	-	rho = -0.454, p = 0.029	-
Total % SFA**	r = 0.504, p = 0.014	-	r = 0.547, P = 0.007	-	-
% C20:2n-6**	-	-	-	r = -0.506, p = 0.014	r = -0.46, p = 0.027
% C20:3n-6**	-	rho = -0.439, p = 0.036	-	-	-

*Value ascertained from dietary analysis
**Value ascertained from GC-FID
r = Pearson's correlation coefficient; rho = Spearman's rank correlation coefficient
Abbreviations: ARP = Absolute refractory period; circ. – circumference; MUFA = Monounsaturated fatty acid; SFA = Saturated fatty acid; TFA = Trans fatty acid.

Atrial depolarisation demonstrated some significant positive associations with markers of obesity (weight and hip circumference), as well as the proportion of SFA found in capillary blood.

C20:3n-6 (DGLA) was shown to inversely correlate with P wave duration. Both QT and ARP showed negative associations with the dietary intakes of various macronutrients, including the

total amounts of carbohydrate, protein and SFA consumed, and with one capillary blood FA, C20:2n6 (EDA).

From Tables 9 and 10 it is clear that all but 1 of the ECG parameters (P wave duration) were in some way significantly correlated with some measurement of SFA, and none of the ECG parameters correlated significantly with total PUFA intakes, proportion of energy taken in as PUFA, or the proportion of total PUFA found in capillary blood. In addition, no significant relationships were seen between ECG readings and any measurement of total n-6 or total n-3.

Blood pressure

The proportions of certain SFAs and MUFAs quantified by GC-FID showed interesting associations with blood pressure readings (Table 11). The shortest of the LC-SFAs, C14:0 (myristic acid), was associated with an increase in SBP, DBP and MAP. C18:0, C20:0 and the very-long-chain SFAs (VLC-SFAs) C22:0 and C24:0, were inversely associated with blood pressure. The n-7 MUFA C16:1n-7 (palmitoleic acid) had a positive association with blood pressure, while the n-9 MUFA C24:1n-9 (nervonic acid - NA) correlated negatively with all measures of blood pressure.

Table 11. Statistically significant correlations between individual FAs quantified using GC-FID and measures of blood pressure.

	SBP	DBP	MAP
C14:0	r = 0.51, p = 0.013	rho = 0.633, p = <0.001	rho = 0.66, p = <0.001
C18:0	rho = -0.456, p = 0.029	rho = -0.651, p = <0.001	rho = -0.605, p = 0.002
C20:0	r = -0.477, p = 0.021	r = -0.583, p = 0.004	r = -0.595, p = 0.003
C22:0	-	r = -0.442, p = 0.035	r = -0.429, p = 0.041
C24:0	-	r = -0.709, p = <0.001	r = -0.643, p = <0.001
C16:1n-7	rho = 0.533, p = 0.009	rho = 0.512, p = 0.013	rho = 0.586, p = 0.003
C24:1n-9	r = -0463, p = 0.026	r = -0.707, p = <0.001	r = -0.672, p = <0.001

r = Pearson's correlation coefficient; rho = Spearman's rank correlation coefficient
Abbreviations: DBP = Diastolic blood pressure; FA = Fatty acid; GC-FID = Gas chromatography - flame ionisation detection; MAP = Mean arterial pressure; SBP = Systolic blood pressure.

Daily macronutrient intakes as a percentage of total energy

The mean daily intakes of the main macronutrients as a percentage of total energy, as calculated in Table 6, were correlated with each other to look for any significant relationships that could be used to make further inferences with regard to associations seen elsewhere. The correlation matrix is given in Table 12, with the significant associations shown in grey.

Table 12. Correlation matrix for the percentages of energy consumed as the different macronutrients

	% of energy as carbohydrate	% of energy as protein	% of energy as fat	% of energy as SFA	% of energy as MUFA	% of energy as PUFA
% of energy as protein	rho=-0.434; p=0.04	-				
% of energy as fat	rho=-0.479; p=0.022	rho=-0.107; p=0.627	-			
% of energy as SFA	rho=-0.196; p=0.369	rho=-0.102, p=0.643	rho=0.553, p=0.007	-		
% of energy as MUFA	rho=-0.415; p=0.05	rho=0.107; p=0.627	rho=0.535, p=0.01	rho=0.131, p=0.548	-	
% of energy as PUFA	rho=-0.42; p=0.047	r=0.014, p=0.949	rho=0.606, p=0.003	rho=0.004, p=0.987	r=0.786, p=<0.001	-
% of energy as TFA	rho=-0.25, p=0.249	r=-0.22, p=0.314	rho=0.168, p=0.442	rho=0.122, p=0.579	r=0.467, p=0.025	r=0.286, p=0.185

r = Pearson's correlation coefficient; rho = Spearman's rank correlation coefficient.
Results in grey are those where statistical significance was achieved.
Abbreviations: MUFA = Monounsaturated fatty acid; PUFA = Polyunsaturated fatty acid; SFA = Saturated fatty acid; TFA = Trans fatty acid

Carbohydrate intake as a percentage of daily energy was found to negatively associate with protein, total fat, MUFA and PUFA intakes. The percentage of daily energy intake as fat was positively associated with SFA, MUFA and PUFA intake, but not with TFA. Percentage of daily energy taken in an MUFA correlated positively with both PUFA intake and TFA intake.

BMI variability

Consideration was given to the fact that the wide variability in BMIs recorded could have affected results as BMI is a strong marker of obesity, which has effects on the heart. Therefore, to check whether BMI could have been a significant confounder, a final set of correlations was performed. This involved dividing the participants up into two groups according to their BMI and correlating these BMI readings with all of the ECG parameters (Table 13). One group consisted of those who were in the underweight (<18.5) and ideal (18.5 to <25) BMI categories (n = 13), and the second group was made up from those whose BMIs put them in the overweight (25 to <30) and obese (>30) categories (n = 10). A statistically significant positive correlation was discovered between the QRS duration and the BMI of the underweight/ideal group, but not with the overweight/obese group.

Table 13. Correlations between all ECG phases and the two separated BMI groups

	Underweight and normal BMI	Overweight and obese BMI
AUC of QRS (mm^2)	r = 0.163, p = 0.594	r = 0.271, p = 0.449
QRS duration (ms)	r = 0.625, p = 0.022	r = 0.207, p = 0.567
R wave amplitude (mV)	r = -0.037, p = 0.903	R = 0.364, p = 0.301
PR interval (ms)	r = 0.181, p = 0.555	r = 0.013, p = 0.971
P wave duration (ms)	rho = 0.481, p = 0.096	r = 0.13, p = 0.721
P wave amplitude (mV)	r = -0.18, p = 0.557	rho = 0.6, p = 0.073
QT duration (ms)	r = 0.153, p = 0.618	r = 0.059, p = 0.872
ARP (ms)	r = 0.097, p = 0.753	r = -0.121, p = 0.74

r = Pearson's correlation coefficient; rho = Spearman's rank correlation coefficient
Abbreviations: ARP = Absolute refractory period; AUC = Area under the curve; BMI: Body mass index.

DISCUSSION

Anthropometric data

The average body fat percentage of this group (36%) was higher than the recommended level, which, for women between the ages of 20 and 39, is 21-32% (Gallagher *et al.,* 2000). The mean BMI, at 25.79, was marginally into the overweight category as a healthy BMI is between 18.5 and 25 (WHO, 1995 and 2000). 43% of the individuals (10/23) had a BMI higher than 25, and around 40% of the global population is considered to be overweight or obese (WHO, 2021), so the study group was very close to being representative of the general population in this respect, but the potential confounding effect of the varied and elevated BMI on the study outcome was considered later.

Mean waist circumference was 80.04cm, which was marginally more than the 80cm recommended to reduce risk of CVD (Lean *et al.,* 1995). In fact, more than half of participants (12/23) had a waist circumference greater than 80cm, indicating undesirable levels of abdominal adiposity, and consequently a greater risk of CVD-related mortality (Zhang *et al.,* 2008). Waist to hip ratio is also considered a valid marker of health status and central obesity (Ibrahim & Ahsan, 2019), and a more appropriate predictor of all-cause mortality than BMI (Srikanthan *et al.,* 2009). Considering the mean waist to hip ratio was 0.77 within this group, which is just below the WHO recommended cut-off point of 0.85 (WHO, 2008), this was suggestive of a relatively healthy study population. This health status was further corroborated by the mean levels for haematocrit, heart rate, and oxygen saturation, which were all within normal limits.

Normal blood pressure should be between 90/60mmHg and 120/80mmHg (SBP/DBP) and in this test group, mean SBP was 117.52 and mean DBP was 76.96, with some participants having high readings for both. A normal MAP reading should be between 70 and 100mmHg, which the average ascertained here was, although, at 90mmHg, it is considered to be on the high side of normal (Melgarejo *et al.,* 2021). According to the 2019 Health Survey for England (NHS Digital, 2020) blood pressures within the general population tend towards the higher side of normal, with around 11% of adult women in the UK having untreated hypertension. It should be pointed out, however, that blood pressure is also a marker of emotional state, and it can be artificially raised when being taken in a clinical setting due to 'white coat syndrome' (Pioli *et al.,* 2018), so this slight elevation in some readings was not a great cause for concern. Other factors that could have had a confounding effect on blood pressure readings are considered in the limitations section, but no participant was eliminated for a high blood pressure reading as the main objective of the study

was to consider the potential effect of PUFA intakes on ventricular depolarisation, with blood pressure included to potentially provide additional insight.

Taking all this into account, and given the exclusion criteria that were applied as participants were recruited, it is fair to say that the study population, although small, was a fairly good representative sample of women aged between 25 and 40 from the general population.

Nutritics – Dietary analysis

At only 1577kcal, the average energy intake for this group was around 400kcal less than is recommended for women in this age group (approx. 2000kcal) (DoH, 1991). Although surprising, considering this population is bordering on overweight, it has been demonstrated that under-reporting of food consumed, or temporary modification of diet, due to embarrassment, guilt or inconvenience is common in dietary analysis (Macdiarmid & Blundell, 1997), so the actual calorific intake was likely to have been higher. It should be noted that 2 participants reported daily energy intakes of around 700-800kcal, which is considered a very low-calorie diet (Joshi & Mohan, 2018). However, taking into account this general tendency to under-report, it was decided to leave these participants in the study as their data could still contribute to any relationships witnessed between intake proportions and ECG markers.

The study group reported eating a greater percentage of both protein and fat (18% and 37% respectively) (Table 6) than is ideal, resulting in a slightly lower than recommended carbohydrate intake as a percentage of total energy consumed. Compared with what is recommended, participants reported consuming a larger proportion of their total fat as SFA (13% as opposed to 10%), which is not advised as SFA is known to have numerous negative health effects (Zhou *et al.,* 2020; Zhuang *et al.,* 2019). However, due to the low average energy intake, the mean total SFA consumed, by weight, was still slightly less (23.41g) than the 24g daily limit set for women (DoH, 1991). Both MUFA and PUFA intakes as a percentage of total energy intake were around 3% and 2% lower, respectively, than is advised. It is recommended that of the PUFA consumed, between 1.4 and 2.5g per day should constitute the n:3 family (Molendi-Coste *et al.,* 2011), which, at an average of 0.9g/day, this study group fell well below. Conversely, and more encouragingly, the intake of harmful TFA in this group was less than half a gram, and 0.25% of total energy intake, which is far from the upper limit of 2% of total energy (DoH, 1991).

ECG analysis

Average measurements from the ECG read-outs of all participants demonstrated that all but one of the recorded parameters were within normal limits where those are stated. There is no

expected value for the AUC of the QRS in the literature yet as it has only been recognised as a valuable ECG parameter in recent years (Van Stipdonk *et al.,* 2018). ARP was the only parameter where the mean value was prolonged, compared to what is considered normal. This could be attributed to a consistent error in the way this measurement was carried out. The fact that all other means were within normal limits was useful in being able to make accurate and meaningful correlations between ECG measurements and other data sets.

Fatty acid analysis – GC-FID

The values for blood fatty acids ascertained by GC-FID compared favourably with what has been observed in other studies (Min *et al.,* 2011), with total percentages of SFA, MUFA, n-6 and n-3 being very close to what is expected in capillary blood samples.

Statistical analysis and discussion on significant correlations

ECG parameters – QRS complex

The three parameters most pertinent to the objective of this study were the AUC of the QRS, QRS duration and R wave amplitude, since they are all different measures of the QRS complex itself, which represents ventricular depolarisation (Jarvis, 2021). Ventricular depolarisation can be delayed in those with ventricular hypertrophy, so a larger AUC, a longer QRS duration and a taller R wave can all indicate myocardial hypertrophy and dysfunction, and the larger, longer and taller they are, the less efficient the propagation of APs and subsequent contraction of cardiac muscle (Bonoris *et al.,* 1978; Murkofsky *et al.,* 1998), which could suggest a higher CVD risk.

The significant positive correlations revealed between two of the ECG parameters (AUC of QRS and R wave amplitude) and dietary intakes of total fat, SFA, total energy and carbohydrate suggest that these ECG measures are most closely linked, and that excessive calorific intake, predominantly in the form of fat and carbohydrates, could be affecting ventricular physiology and function in this sample. Extensive research exists to prove that ventricular hypertrophy is a hallmark of obesity in all ages, including young children (Bartkowiak *et al.,* 2021; Cuspidi *et al.,* 2014), and this study appears to support this point. Although these 2 parameters themselves did not correlate significantly with markers of obesity, the other parameter relating to ventricular depolarisation - duration of the QRS - did (Table 9). Significant associations were seen between it and weight, BMI, waist circumference, and hip circumference. These are all markers of obesity and central adiposity, which is known to increase the risk of CVD and so this completes the connection between high energy intake, obesity and subsequent perturbations in ventricular muscle and function. Taken together, these results show the effect of dietary intakes on obesity,

with subsequent effects on the specific mechanisms involved in ventricular depolarisation. It should be noted that the QRS duration also positively correlated with the reported percentage of energy intake as both fat and SFA (Table 9). Given the additional positive correlation between proportional fat and SFA intakes (Table 12), it would be fair to infer that the negative effect on QRS duration is therefore most probably attributed to SFA. SFAs are complicated compounds, but they are still widely implicated in the progression of CVD due, in part, to the increase in LDL concentration that is associated with high intakes (Jakobsen *et al*., 2009), and it is agreed that intake should be limited in order to reduce risk (Maki *et al.,* 2021).

Both AUC of the QRS and R wave amplitude demonstrated negative correlations with the proportion of energy taken in as protein and the proportion of C18:1n-7 (VA) found in the blood. Regarding protein, it is difficult to surmise whether the higher proportion of protein itself could be having a positive effect on contractility, or, whether it could relate more to the concurrent significant negative correlation between energy taken in as protein and as carbohydrates (Table 12). Evidence can be found to support the idea that a low carbohydrate/high protein diet has beneficial effects on cardiac function in overweight patients (von Bibra *et al.,* 2014), and this result lends support to that finding, but cannot explain the mechanism.

With regard to VA, this is a MUFA which can be found in both *cis*- and *trans*- forms, and although there is no recognised association between it and the risk of CVD (Field *et al.,* 2009), it is a precursor to conjugated linoleic acid (CLA), which is itself an isomer of LA. Some isomers of CLA have been shown to slow the progression, and even bring about regression, of atherosclerotic plaques that can lead to ischemic events (Bruen *et al.,* 2017; Stachowska *et al.,* 2012). Atherosclerosis can both cause, and be caused by, hypertension, and both are factors in the increased ventricular workload that eventually leads to an increase in ventricular muscle mass, which can then be visualised in the QRS complex of the ECG (Kahan & Bergfeldt, 2005). The negative association between VA and AUC of the QRS could therefore be tentatively attributed to the connection between VA and CLA.

Arguably the most noteworthy correlations established in relation to ventricular depolarisation are the negative association between the AUC of the QRS and the amount of C22:6n-3 (DHA) as a percentage of total blood FAs, and the positive associations between the n-6:n-3 ratio of blood FA composition as determined through GC-FID, and both the AUC of the QRS and R wave amplitude. These relationships add weight to the already well-established ideas that DHA is particularly beneficial to the heart and its function (Leaf *et al.,* 2003), and that the ratio of n-6:n-3 is an important dietary consideration in reducing the risk of CVD (Simopoulos, 2006).

In this study there is a clear indication that as DHA increases, AUC decreases, which suggests that DHA is somehow enabling more efficient AP propagation through the ventricular tissue. This study cannot point to a particular mechanism involved, however, there is plenty of research that could help to explain this finding. PUFAs of the n-3 family, and particularly EPA and DHA, are known to interact with ion channels in cardiomyocyte membranes in such a way that AP duration is reduced which, in healthy individuals with no recorded cardiovascular issues, is a good thing (Moreno *et al.,* 2012). Studies investigating the relationship between the intake of fish oil, a particularly good source of n-3 PUFAs, and the incidence of arrhythmia find that n-3s are capable of stabilising the electrical activity in cardiomyocytes (Tribulova *et al.,* 2017; Xin *et al.,* 2013), and it is also recognised that dietary supplementation with n-3s can attenuate enlargement of the left ventricle, thus improving contractile function (Duda *et al.,* 2007).

With regard to the PUFA ratio, as previously mentioned, during an immune reaction both n-3 and n-6 PUFAs are involved in the cells' inflammatory response and compete for the same enzymes in the production of both pro- and anti-inflammatory molecules. As n-6 PUFAs tend to produce more pro-inflammatory metabolites, and n-3 are considered anti-inflammatory, a long-term imbalance in the intake of n-6 and n-3, results in a chronic inflammatory state (Calder, 2017). Although some level of inflammation is needed in an immune response, long-lasting, low-level inflammation can damage the cells of the endothelium, which can lead to atherosclerosis, hypertension and a reduction in function (Nishida & Otsu, 2017). The associations seen in this study between n-6:n-3 ratio and the two parameters of ventricular depolarisation agreed with the research in this respect, as some level of ventricular dysfunction caused by high n-6/low n-3 intake is implied through the delay in AP propagation.

Other ECG parameters - P wave

PR interval and P wave duration, which represent atrial depolarisation and the propagation of the AP from the atria to the ventricles via the AVN, respectively, were positively associated with both weight and hip circumference. The implication here is that as markers of adiposity increase, so does the time taken for the AP to travel through the atrial myocardium. As with ventricular tissue, this delay is likely caused by hypertrophy, which can be brought about by chronic hypertension (Kockskämper & Pluteanu, 2022), a well-known risk factor for CVD, which can itself be caused by obesity (Jiang *et al.,* 2016). Additionally, there is a significant positive correlation between the proportion of capillary FAs that are SFA, and both PR interval and P wave amplitude. This again, adds weight to the argument that SFA is associated with CVD, probably through its contribution to obesity (Zhou *et al.,* 2020).

A significant negative association was established between P wave duration and the percentage of the individual FA C20:3n-6 (DGLA) identified in capillary blood by GC-FID. Although n-6 FAs are typically deemed to be pro-inflammatory, DGLA in particular is believed to form metabolites that bestow anti-inflammatory properties and reduce atherosclerosis (Fan & Chapkin, 1998; Wang *et al.,* 2012), which would explain why higher levels were related to a decreased P wave duration in this investigation.

Other ECG parameters - QT duration and ARP

QT duration is representative of the ventricular depolarisation and repolarisation phases and is known to be prolonged in those with obesity (Omran *et al.,* 2018). A prolonged QT duration implies a delay in repolarisation and has been associated with an increase in the risk of arrhythmias in ventricular myocytes (Algra *et al.,* 1991; Wheelan *et al.,* 1986). It was surprising to see that in this study a longer QT duration was associated with a lower intake of total calories and individual macronutrients, including SFA and TFA (Table 10), as it could be assumed that those taking in more calories and 'bad' fats per meal would be the individuals most likely to be overweight and therefore have prolonged QT durations. However, also in this group, few positive associations were established between daily intakes of any of the macronutrients and markers of obesity such as body fat %, BMI and waist:hip ratio (Appendix D, Tables D28-D31), so no such assumption can be made with this sample.

A similar relationship was observed between ARP and various macronutrient intakes, explained by the fact that ARP and QT duration are very closely related. The surprising relationships observed between certain macronutrient intakes and both QT duration and ARP could be explained by the 'white coat phenomenon' already discussed (Pioli *et al.,* 2018). In a clinical setting, if the heart rate is raised, this could falsely reduce QT duration (Viitasalo & Karjalianen, 1992), so it would be advisable in future study to correct the QT duration for heart rate before correlations are made, using Bazett's heart rate correction formula, or Fredericia's formula (Davey, 2002). Heart rate could also be measured more than once and then averaged.

MUFA intake was negatively correlated with QT duration, suggestive of a positive effect of dietary MUFAs on this phase of the cardiac cycle. Interestingly, there was also a positive correlation between % of energy taken in as MUFA, and the % of energy taken in as PUFA (Table 7). Although PUFA intake itself did not correlate with QT duration, or indeed any aspect of the ECG, when these two significant associations are taken together, it supports the belief that those individuals who consume a greater proportion of their energy from FAs as 'good' fats are experiencing benefits to heart health. MUFAs are a controversial set of FAs due to the variety of dietary

sources from which they can be obtained, which is often not taken into account when studying their health effects (Guasch-Ferré *et al.*, 2019). Different MUFAs can be obtained from both plants and animals, and unfortunately the results from this study cannot separate the two. However, in general they are believed to play a part in increasing levels of beneficial HDL cholesterol, although the mechanisms by which this occurs have only been speculated (Cao *et al.,* 2022). MUFAs are also thought to assist in reducing plasma triglyceride levels (Cao *et al.,* 2022), with high triglyceride levels being a known risk factor in the development of CVD (Sarwar *et al.,* 2007).

The only specific FA found in capillary blood to significantly correlate with QT duration and ARP was C20:2n6 (EDA), an n-6 PUFA that is an elongation product of LA, and a precursor to DGLA and AA (Huang *et al.,* 2011). Negative relationships were seen in both cases meaning that as EDA in the blood increased, QT duration and ARP reduced. Given the association between arrhythmia and a longer QT duration, it could be inferred that EDA is having a positive effect on the ventricular muscle cells. However, when considered from the angle that those with a long QT duration and ARP have a smaller proportion of EDA in their blood, it could simply imply that, as opposed to a causational correlation between EDA and ventricular function, these individuals are possibly consuming less LA, and that this is having a negative effect on the heart. LA is thought to have some beneficial effects on blood lipid profile through its involvement in reducing the amount of harmful very low density lipoprotein (VLDL) and LDL cholesterol and increasing the amount of HDL cholesterol in circulation (Froyen & Burns-Whitmore, 2020), so this could be a possibility. However, a significant negative relationship was also seen between LA and EDA (Appendix D, Table D52 : $r = -0.445$, $P = 0.034$), and no correlation was seen between a longer QT/ARP and either the dietary intake or the capillary blood levels of other PUFAs (Appendix D, Tables D7 & D8), so inferring what the observed relationship between EDA and QT/ARP could signify is problematic. It is highly likely, given the very small proportion of total blood FAs that EDA makes up, and the complexity of metabolic pathways that FAs can take within the body, that this relationship is not of vital importance.

ECG parameters and general FA intake

Looking at the ECG parameters as a whole, it was interesting to observe that at least one measure of SFA, whether it be dietary intake, percentage of energy as SFA, or proportion of blood FAs as SFA, was correlated with each of the ECG measures with the exception of P wave duration. This further supports the argument that SFA is likely to affect the function of the heart in some way, with large prospective cohort studies confirming that this affect is indeed negative. Zong *et al.*

(2016) found that the LC-SFAs (those containing more than 12 carbons) were much more harmful to cardiovascular health than the short and medium-chain SFAs (SC-SFAs and MC-SFAs), and that they should be greatly reduced in the diet in favour of either PUFAs, MUFAs, whole grain carbohydrates or plant-derived proteins in order to lower the risk of CVD.

It was also interesting to note that no relationships were seen between total PUFA intake, percentage of energy as PUFA or proportion of blood FAs as PUFA and any of the ECG parameters in this study. Neither were any relationships discovered between any of the measurements of total n-6 PUFA and ECG parameters. There is still some controversy surrounding n-6 PUFAs and their effect on the heart due to their known connection with inflammatory states, but there is some evidence to suggest that they can influence blood lipid profile by helping to increase hepatic LDL receptor activity (Fernandez & West, 2005). This results in a reduction in the amount of circulating LDL, which a recent and novel study utilising Mendelian randomisation analysis suggests has an effect on ventricular mass. Aung *et al.* (2020) discovered that, as well as having a role to play in the formation of atherosclerotic plaques, LDL cholesterol may also show a causal relationship with remodelling of ventricular structure in such as way that it increases the risk of CVD. This could therefore complete a connection between n-6 PUFAs and ventricular hypertrophy, but which was sadly not identified in the course of this study.

More surprising still was the fact that no relationships were seen between any of the measures of total n-3 PUFA and any of the ECG markers. As n-3 are considered the PUFA most beneficial to human health, it was hoped that this study could have better supported their cardioprotective contribution. EPA and DHA, in particular, are known to reduce the risk of CVD through the benefits of having them incorporated into membrane lipids, and their involvement in certain signal transmission pathways (Hulbert *et al.,* 2005). They also have significant, advantageous effects on the expression, in cardiomyocytes, of genes involved in processes such as inflammation (interleukin-6), angiogenesis (transcription factor-19), ion movement (caveolin-2) and cell survival (angiopoietin-2) (Bordoni *et al.,* 2007). Interleukin-6 and transcription factor-19 are both implicated in ventricular hypertrophy. This could explain the one relationship that was highlighted in this study, between an n-3 PUFA (DHA) and AUC of the QRS.

Blood pressure

Although not central to the original question posed by this study, it was thought-provoking to witness a number of relationships between a few of the individual SFAs and MUFAs identified through GC-FID and the measurements of blood pressure (SBP, DBP and MAP). High blood pressure, often caused by high cholesterol, is a major factor involved in the progression of CVD

(Fuchs & Whelton, 2020), and is known to be a direct cause of ventricular hypertrophy as the abnormal workloads within the chambers cause changes in cardiac morphology (Lovic *et al.,* 2017). Relationships seen here could therefore have implications for the roles of certain FAs in ventricular depolarisation, despite not correlating with the relevant ECG parameters themselves.

Some outcomes from the current study agree with findings from Simon *et al.* (1996), who reported negative correlations between C18:0 (stearic acid) and blood pressure readings, and positive associations between C16:1n-7 (palmitoleic acid) and both SBP and DBP. A recent study in support of this link between stearic acid and markers of CVD claimed that stearic acid lowers LDL cholesterol, a risk factor for CVD and a known cause of hypertension (Van Rooijen & Mensink, 2020). However, studies that show no association between stearic acid and blood lipid profile have also been published (Flock & Kris-Etheron, 2013; Mensink *et al.,* 2003), as well as studies claiming that stearic acid increases the risk of CVD (Praagman *et al.,* 2018; Zong *et al.,* 2016). The only deduction to be made from this at present is that the actual role that stearic acid might play in blood lipid profile and associated hypertension that could indicate a CVD risk is not yet fully understood.

In contrast to the positive associations between palmitoleic acid and blood pressure readings seen in both this study, and from Simon *et al.* (1996), Tang *et al.,* (2021) reported that palmitoleic acid showed a negative correlation with blood pressure in a large sample of children and adolescents. An additional animal study described in the same article by Tang and colleagues deduced that the effect of palmitoleic acid on blood pressure is brought about though its inhibition of an inflammatory response that is mediated by a protein called NFκB - nuclear transcription factor-kappa B.

The SFA C14:0 (myristic acid), as the shortest of the LC-SFAs, is thought to be involved in raising LDL cholesterol levels by negatively affecting the activity of LDL receptors (Fernandez & West, 2005). This study supports that implication, as C14:0 was seen to associate positively with all blood pressure measures.

A large cohort study reported that higher levels of circulating C20:0 (arachidic acid), C22:0 (behenic acid), and C24:0 (lignoceric acid) – the very-long-chain SFAs (VLC-SFAs) - are associated with a lower risk of heart failure (Lemaitre *et al.,* 2018). This would tend to agree with the findings from the current study, which saw these same SFAs correlate negatively with blood pressure readings, implying that these SFAs possess cardioprotective properties.

There is very little in the published literature about nervonic acid (NA) and any connection it may have with cardiovascular issues. It is known to be involved in myelin synthesis, so has an important role to play in the brain (Li *et al.,* 2019). A recent study by Pellegrini *et al.* (2021) showed that serum levels of NA were positively correlated with an increased risk of atrial fibrillation in older adults, and a 2005 study reported that negative correlations were seen between NA and six different risk factors for CVD, including BMI, fasting blood sugar levels, total cholesterol and triglyceride levels (Oda *et al,.* 2005). Although Oda and colleagues found no specific associations between NA and blood pressure readings, the relationships that were discovered were still indicative of a cardioprotective function, which the current study would corroborate.

BMI variability

It was discovered that the wide variability in the BMI values for this sample could have been affecting some results as, when split into two separate groups, a positive association was found between those with a low to ideal BMI and the duration of the QRS, but not with the overweight and obese category. Although the BMIs of the sample in its entirety (n = 23) was already shown to correlate positively with QRS duration, the fact that on reducing the BMI variability that same relationship was only seen in one of the groups, proves that BMI could have been a confounding variable. Thus, if this study were to be replicated, it would be advised to use selection criteria that takes this into account and to recruit only participants from one or two of the BMI categories. This would ensure greater accuracy and value in any significant correlations that were then seen between other variables.

General discussion

Some anthropometric biomarkers of obesity were found to be associated with delays in the ventricular depolarisation and repolarisation phases of the cardiac conduction system, which highlights the known connection between obesity and ventricular hypertrophy (Cuspidi *et al.,* 2014). Ventricular hypertrophy is enlargement of muscle mass that occurs when there is a chronically increased effort experienced in that muscle, usually as a consequence of high blood pressure (Lorell & Carabello, 2000). Obesity is considered one of the major risk factors in the development of heart disease, both independently, and via its connection with a whole host of CVD comorbidities which includes hypertension, amongst others (Powell-Wiley *et al.,* 2021). Almost 2 billion adults worldwide were classed as overweight or obese in 2016 (WHO, 2021), and in 2015 BMI was thought to be a causative factor in around 4 million deaths, with CVD also implicated in around 70% of those (Afshin *et al.,* 2017). Not all overweight or obese people have

CVD, and not all people with CVD are overweight or obese, but the connection is difficult to ignore. In cardiac rehabilitation settings it has been reported that more than 80% of patients seen were in the overweight category, and almost half were considered obese (Audelin *et al.,* 2008). The degree of weight loss that can be achieved through bariatric surgery has been shown to greatly reduce the risk of heart disease (Batsis *et al.,* 2008), and in an earlier study, weight loss was seen to have a beneficial effect on ventricular function (Karason *et al.,* 1998). This is all proof that one way in which obesity contributes to CVD is through the detrimental effects on the ventricular muscle, which was also recognised in this study.

Certain macronutrient intakes, particularly SFA, were shown to consistently associate with different parts of the cardiac cycle in a detrimental manner. However, in researching the effect of SFA on the heart, it becomes obvious that saturated fats should not all be tarred with the same brush, and this can be supported by the positive effects some of the LC-SFAs were seen to have on blood pressure readings in this study.

The biological properties and functions of SFAs tend to vary according to their length. SC-SFAs (<6 carbons) are endogenously-produced as required via colonic fermentation of the more complex dietary carbohydrates (Hellerstein, 1999). These are thought to then play a part in the expression of genes involved in a number of metabolic and anti-inflammatory processes (Tan *et al.,* 2014). The MC-SFAs (6-12 carbons) tend to be oxidised for fuel and so do not show great associations with CVD risk (Ruiz-Nuñez *et al.,* 2016). The VLC-SFAs (>20 carbons) are reported to have advantageous effects on healthy aging, which includes preventing the development of CVD (Bockus *et al.,* 2021). Which leaves the LC-SFAs of 14 to 20 carbon atoms, often found in ultra-processed foods such as baked goods, ice creams and ready-meals (Houston, 2018). Ceramide molecules, which are often involved in both inflammation and programmed cell death and are implicated in some CVD risk markers, incorporate SFA molecules into their structure (Chaurasia & Summers, 2015). Ceramides comprising LC-SFAs such as palmitic acid (C16:0) are thought to contribute to these negative health effects, but ceramides incorporating VLC-SFAs are believed to have the opposite effect (Grösch *et al.,* 2012). The proportion of total fatty acids found in human tissues that are SFAs is between 30% and 40%, with the majority of that (up to 25% of total) being palmitic acid (Min *et al.,* 2011). Palmitic acid has been associated with an increased risk in various aspects of CVD, including atrial fibrillation (Fretts *et al.,* 2014). However, the next most abundant SFA in tissues, stearic acid (C18:0), shows no such correlation, and in some studies has even been shown to reduce CVD risk markers such as LDL cholesterol (Hunter *et al.,* 2010; Van Rooijen & Mensink, 2020). It could be said therefore that the reputation of SFAs as a general class of

harmful molecules has been largely formed on the basis of palmitic acid's unfavourable biological effects. Nevertheless, considering palmitic acid's abundance in the human diet and its accumulation in tissues, it would seem prudent to follow the general recommendations with regards to reducing overall SFA intake, especially considering that popular modern processed foods high in SFAs also tend to contain more TFAs, fewer n-3 PUFAs and low-quality carbohydrates (Okręglicka, 2015). Indeed, studies looking into SFA intake and CVD in whole populations have witnessed that those groups with a lower incidence of CVD are taking in less dietary SFA, and those that see no association between high SFA intake and CVD are taking in less TFA and a higher intake of n-3 PUFAs (Ruiz-Nuñez *et al.,* 2016).

Despite MUFAs, PUFAs, n-6 or n-3 FAs not demonstrating any general associations with different phases of the ECG, some individual blood FAs were related to different aspects of the cardiac conduction system in ways that could be suggestive of both negative and positive effects on the heart. Most of these FAs occur in such small proportions in the individuals in this study (0.1 – 1.5 % of total FAs) that it would be unwise to suggest direct and causational relationships as the outcome of such as a small investigation. However, the relationship between DHA, which made up closer to 3% of total FAs, and the AUC of the QRS is worth a mention in relation to the evidence already in existence that suggests a beneficial effect.

The significant negative relationship between DHA and AUC of the QRS is suggesting an important role for DHA in the electrical stabilisation of, and the propagation of APs through, the ventricular muscle tissue. It has been reported previously that n-3 PUFAs have a preventative role to play in ventricular fibrillation, a state which is brought about through untimely excitation of partially depolarised myocytes (Billman *et al.,* 1999; Christensen, 2003), and which can precede sudden cardiac death (Albert, 2002). The process by which this happens is most likely due to the specific effect that EPA and DHA have on ion channel function. As detailed in the introductory section, APs are propagated through cardiomyocytes thanks to a complicated course of action involving the movement of ions across cell membranes. For this process to occur properly, ion channels must allow ions to move freely as appropriately needed. Membrane phospholipids that incorporate EPA and DHA alter the morphology of the membrane in such a way that it opens up the channels, allowing greater freedom of movement (Leaf *et al.,* 2003). It has also been noted that n-3s in membranes are capable of restricting the activity of L-type Ca^{2+} channels, the result of which is the prevention of too much calcium entering the cells too rapidly which would inadvertently set off the contractile process – termed a 'triggered arrhythmia' (Billman *et al.,* 1999; Xiao *et al.,* 1997). Additionally, EPA and DHA suppress the activity of voltage-dependent Na^{+} channels, resulting in an extension in the time that these channels are inactivated, which

prevents an AP from being propagated too soon – known as an ischaemia-induced arrhythmia (Billman *et al.,* 1999; Xiao *et al.,* 1995).

The beneficial action of EPA and DHA in these processes is thought to be enabled through the formation of lipid rafts. Lipid rafts are ordered assemblies of lipids, termed 'microdomains', in the plasma membrane, that allow efficient compartmentalisation and subsequent enhancement in function of membrane signalling molecules and ion channels (Ouweneel *et al.,* 2020). It is when n-3 PUFA molecules are incorporated into lipid rafts, in favour of cholesterol, that membrane protein function is believed to be most improved (Shaikh, 2012). Interestingly, it is thought that membrane proteins with specific roles in inflammation are particularly profuse within lipid rafts (Garattini, 2007).

As well as being anti-arrhythmic, PUFAs of the n-3 family are involved in a number of other valuable outcomes for cardiac health that could provoke an effect on the AUC of the QRS. As discussed already, when ventricular hypertrophy occurs, this can have a measurable effect on the QRS complex. Hypertension is accepted as the predominant causative factor in hypertrophy, although other factors can play a part (Kahan & Bergfeldt, 2005), and n-3 PUFAs can exert anti-hypertensive actions via their roles in atherosclerosis, thrombosis formation and inflammation.

Focusing first on thrombogenesis, early investigations into the low incidence of CVD in Greenland Eskimos demonstrated that those with a high n-3 intake from seafood sources experienced fewer blood clots (Dyerberg & Bang, 1979), but also more haemorrhagic events due to n-3 PUFA's involvement in reducing blood viscosity (Bang & Dyerberg, 1980). Following these studies, DHA and EPA were awarded their anti-thrombogenic titles, although the mechanisms of action remained unknown. It has since been discovered that the more n-3 PUFAs there are incorporated into membrane phospholipids, the less thromboxane A2 (TXA2) is produced, which is a molecule key to platelet aggregation (Kramer *et al.,* 1996). Other roles for n-3 PUFAs in platelet activation and adhesion have also been identified (Phang *et al.,* 2013), with the overall outcome from these actions of DHA and EPA being less viscous blood, which is easier for the heart to pump around the body. The more viscous the blood, the greater the effect on blood pressure (Fowkes *et al.,* 1993), which explains one method by which n-3 PUFAs can modify hypertension.

With regard to the anti-atherogenic properties of n-3 PUFAs, autopsy studies allowed comparison of atherosclerotic lesions in populations with high n-3 intake versus populations with low n-3 intake, with fewer lesions seen in those who consumed more n-3 PUFAs from seafood sources (Newman *et al.,* 1993). This meant that the anti-atherogenic contributions of EPA and DHA could be alluded to before a mechanism of action could be explained. To understand the ways in which

n-3 can affect the progression of atherosclerosis, is it important to understand how atheroma are formed. High cholesterol, particularly of the VLDL variety, high levels of circulating triglycerides, and high blood pressure can all result in damage to the cardiovascular endothelium. This damage can attract monocyte cells, and these begin to incorporate themselves, as well as molecules of LDL cholesterol, into the area of damage (De Caterina & Zampolli, 2006). Pro-inflammatory cytokine molecules are also produced by immune cells at the site, which then perpetuates a cycle of inflammation and damage, eventually leading to the formation of an atheroma, the consequence of which can be a heart attack (Falk *et al.,* 1995). PUFAs from the n-3 family can intervene favourably in this process at a number of different points. To begin with, n-3s can reduce the hypertension, high cholesterol and high triglyceride levels that initiate the whole process, via the effects they have on the inhibition of triglyceride and LDL/VLDL synthesis and the increase in catabolism of these molecules (Connor *et al.,* 1993). N-3 PUFAs can also inhibit the action of an important factor involved in endothelial damage and dysfunction, namely NFkB, which is also a key molecule in the body's inflammatory response (Collins, 1993). Additionally, n-3 PUFAs are important in reducing the amount of pro-inflammatory cytokines produced in an immune response, through competitive inhibition (Calder, 2017).

Although these anti-arrhythmic, anti-thrombotic and anti-atherosclerotic effects are important ways in which n-3 PUFAs play a part in the reduction in CVD risk, arguably the most powerful effect is felt in relation to inflammation. An in-depth explanation of the complexities of inflammatory actions within the human body and their inter-relatedness with thrombotic, atherosclerotic and arrhythmic processes besides what has already been examined, is beyond the scope of this study. The known ways in which n-3 PUFAs are understood to reduce chronic inflammation and its harmful consequences are also too numerous to detail here, but these have been widely reported (Calder, 2017; Calvo *et al.,* 2017; Ishihara *et al.,* 2019; Wang & Huang, 2015). Generally, the n-3 PUFAs have a preventative or dampening effect on inflammation through their interaction with cell signalling processes and gene expression (Calder, 2017). The most appropriate mechanism to include in this discussion, as the effect was alluded to in the final significant association that this study identified, is the role n-3 PUFAs play in the competitive inhibition of AA-derived inflammation-promoting molecules.

The ratio of n-6:n-3 PUFAs was found to positively associate with AUC of the QRS and R wave amplitude (Table 9), indicating that an imbalance of these essential PUFAs in favour of n-6 was interfering with ventricular depolarisation. The negative effect of a high n-6:n-3 ratio on cardiovascular health and function is well reported (Simopoulos, 2006) and well understood, and

the mechanism involved, although mentioned in the introductory section, deserves reiteration and further detail.

In response to an inflammatory stimulus, LC-PUFA molecules of both the n-6 and n-3 families are released from their positions in the cell membranes and react with enzymes, including LOX and COX, to form a range of eicosanoid molecules (Calder, 2017). These eicosanoid molecules are then involved in the release of chemicals classed as cytokines, which have varied effects. Cytokines, which include chemokines, interleukins, lymphokines, tumour necrosis factors and interferons, all have special roles to play in the body's inflammatory immune response, with different functions and targets (Ramani *et al.,* 2015). The problem comes when too many of the harmful cytokines are in action, with not enough of the inflammation-resolving forms, such as interferon-β (IFN-β) or resolvins, available to calm this toxic effect (Sommer & Birklein, 2011). This heightened inflammatory state then contributes to an increase in oxidative stress, which encourages the release of more cytokines, and so the vicious circle perpetuates, resulting in a chronic state of inflammation (Calder, 2017; Zhang & An, 2007). The availability of anti-inflammatory chemicals relies largely on the availability of n-3 PUFAs in the membranes of the immune cells, and as these are essential FAs and so required in the diet to meet the body's needs, dietary intake of sufficient n-3 PUFAs is imperative in the fight against inflammation (Calder, 2017). Modern diets consist of an excess of n-6 PUFAs and a deficiency in the n-3 variety, and since the enzymes required to facilitate the production of eicosanoids are shared by both the n-6 and n-3 pathways, an increase in n-3 intake is not much use unless a simultaneous reduction in n-6 intake is also implemented (Simopoulos, 2006). This idea is widely promoted as a means of combating inflammation and reducing the prevalence of inflammatory diseases such as CVD (Calder, 2017; Harris, 2006; Simopoulos, 2006).

Having considered the factors affecting ventricular function, and the ways in which n-3 PUFAs are good for human health, attempting to imply a specific means by which n-3s, and in particular DHA, could be positively affecting ventricular diastolic function in these studied individuals is unwise. Causation cannot be implied from the results seen in this observational study, however, the positive associations that were identified were interesting and generally in line with already-published literature.

The WHO recommendations for PUFA intakes (FAO/WHO, 2010) - that between 6 and 11% of daily energy should be obtained from PUFA; the ratio of n-6:n-3 PUFA should be no more than 5:1; and the combined daily intake of EPA and DHA should total at least 250mg – are largely being missed in Western populations (Eilander *et al.,* 2015; Simopoulos, 2006). Dietary sources of EPA and DHA are restricted to seafoods such as oily fish and algae (Bezard *et al.,* 1994), and people

are being urged to eat between 1 and 2 portions of oily fish per week to meet the recommended levels to reduce CVD risk (Rimm *et al.,* 2018). However, these foods are not consumed in the same amounts as they were prior to the agricultural revolution (Simopoulos, 2006). Populations with a high EPA/DHA intake through seafood consumption, such as Japan, see fewer instances of sudden cardiac arrest than do Western populations (Harris *et al.,* 2008), and clinical studies where individuals were required to increase intake showed reduced incidence of CVD and associated markers (Mozaffarian *et al.,*2004; Jiang *et al.,* 2021). Mohan *et al.* (2021) saw a reduced risk of CVD events in those patients with diagnosed CVD who ate at least 175g of fish per week (2 portions) and He *et al.* (2004) reported that each 20g per day rise in the consumption of fish saw a 7% reduction in the risk of mortality from CVD (He *et al.,* 2004).

However, the outcomes from n-3 supplementation studies can be surprisingly contradictory. A 2018 meta-analysis of large clinical trials concluded that marine-derived n-3 PUFA supplementation does not reduce the risk of cardiovascular events (Aung *et al.,* 2018), but a systematic review since then has shown that supplementation can be effective in reducing cardiovascular events (Shen *et al.,* 2022). Supplementation is still considered debatable in reducing CVD, but n-3 supplementation has showed positive benefits for other aspects of human health. In an in-depth review, health benefits were witnessed in dialysis patients (Friedman & Moe, 2006), a reduction in symptoms for patients with major depressive disorder was seen in another trial (Grosso *et al.,* 2014), and a recent clinical trial on critically ill COVID-19 patients reported that n-3 supplementation gave improved respiratory and renal function (Doaei *et al.,* 2021). So, supplementation has its values, and the lack of compelling evidence for a benefit of n-3 supplementation for CVD could be due, in part, to the host of additional modifiable factors that can increase the risk of CVD, including inactivity, smoking, and drinking alcohol. Therefore, it can be surmised that increasing intake of n-3 PUFAs should be achieved through an increase in consumption of EPA and DHA-containing foods, such as fish, and implemented in conjunction with changes to other lifestyle factors, such as taking more exercise, not smoking, reducing alcohol intake and other dietary changes, which would have the added effect of also reducing obesity.

If simple dietary and lifestyle changes such as these can be effectively and broadly implemented to significantly reduce both the prevalence and the incidence of CVD then this is an easy and cost-effective way to fight a major disease and save millions of lives, and this should therefore be more vehemently promoted. The more evidence there is to support these dietary options as

valuable interventions, the easier that message becomes to convey, and the more likely the general public will listen and make changes.

Limitations

There were a small number of limitations involved in the study and addressing these for future research could provide more credibility and consequence. One obvious limitation is the small sample size (n = 23) as the risk is greater that some of the associations seen could have been due to chance. Unfortunately, the timing of a global pandemic, and the strict selection criteria were both factors in participant recruitment. In saying that, if this study were to be replicated, it would be useful to implement more stringent exclusion criteria with regards to BMI because, as already discussed, the variety in BMI readings was shown to have a confounding effect.

There are always limitations with methods of dietary analysis as honesty and compliance are required from participants. 4 days is considered sufficient to obtain enough information, while giving the best chance of maintaining motivation (Gersovitz *et al.,* 1978). However, under-reporting of foods consumed is common (Macdiarmid & Blundell, 1997), which was suspected in this study. Nevertheless, self-reporting is a general requirement of most methods of nutritional assessment (Shim *et al.,* 2014), so it is a limitation that cannot easily be avoided.

Blood pressure was taken only once, and mean readings were on the higher side of normal. Blood pressure readings can be highly variable during the course of a day, and although all measurements were taken in a seated position, with participants as relaxed as possible, there could still have been other factors at play which might have affected the readings. White coat syndrome has been mentioned already, and to reduce the effect of this, more than one reading could be taken and those readings averaged. Those with consistently high blood pressure readings could then have been eliminated due to the risk of them having undiagnosed hypertension, which would be a confounding variable. Smokers were not included due to the known effect that smoking can have on a number of CVD markers, including blood pressure (Banks *et al.,* 2019), but both stress levels (Munakata, 2018) and activity levels (Bakker *et al.,* 2018) can also affect blood pressure. Stress can cause an increase, and good general fitness can keep blood pressure within normal limits. Oral contraceptive use is also known to affect blood pressure, with the risk of hypertension increasing with prolonged use (Liu *et al.,* 2017). Therefore, more questions regarding lifestyle, and tighter selection criteria could have accounted for these potential confounders.

QT duration was not corrected for the effect that heart rate can have on it. As mentioned, one of the two known formulas that allow this correction could have been used (Davey, 2002). Had this been done, more significant relationships might have been discovered between this ECG parameter and some of the FA measures.

Conclusion

This study sought the answer to the question: Are anthropometric biomarkers, nutrient intake and blood fatty acid composition associated with the electrical activity of the heart in a sample population of healthy women? This was to support the ever-increasing body of evidence that FAs play important roles in human health, and specifically in the development and possible treatment of cardiovascular issues. CVD is a common problem throughout the world, afflicting around half a billion people, and killing over 18 million each year, so it is a global responsibility to comprehend the processes involved in its development and to find preventative avenues for those at risk, as well as more effective treatment options for those already diagnosed.

The simple answer to the study question is: Yes, *some* anthropometric biomarkers and aspects of nutrient intake and blood fatty acid composition showed some associations with the electrical activity of the heart.

Obesity is a modifiable causative factor in CVD and does have specific effects on ventricular depolarisation that can be observed using ECG, as it was in this study population. Research showing that high SFA intake is associated with CVD was corroborated with this study as a high percentage of SFA intake as total energy, and SFA levels in the blood were associated with a number of ECG parameters in ways that could be indicative of ventricular dysfunction. Proportionate levels of individual SFA in the blood were also related to high blood pressure, another causative factor in the development of CVD.

PUFAs of the n-3 family possess cardioprotective qualities due to the countless positive effects they can have on a number of factors that can lead to CVD. This was witnessed in the significant effect that DHA was seen to have ventricular depolarisation, and although causation cannot be inferred, extensive previous research can support this finding. The clear relationship that already exists between the high n-6:n-3 ratio and inflammation can also be reinforced by this study through the significant association seen between the PUFA ratio and ventricular depolarisation, with inflammation being a known factor in ventricular dysfunction.

This study therefore supports a link between FA intake and cardiac function, corroborating the general recommendations to reduce SFA intake and to increase PUFA intake for the benefit of the heart, but in particular, for improved ventricular function.

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APPENDICES

APPENDIX A

APPLICATION FOR ETHICAL APPROVAL
STAFF, ASSOCIATE RESEARCHER & PG RESEARCH STUDENT*

*(*including PhD, DBA, Professional Doctorate and MRes)*

To be completed by staff, associate researchers and students enrolled on postgraduate research degrees proposing to undertake ANY research involving humans [that is research with living human beings; human beings who have died (cadavers, human remains and body parts); embryos and fetuses, human tissue, DNA and bodily fluids; data and records relating to humans; human burial sites] or animals.

SECTION A: REVIEW PROCESS	
Please indicate which Research Ethics Panel you are submitting your application to:	
COLLEGE OF BUSINESS, PSYCHOLOGY AND SPORT **(CBPS REP)**	☐
COLLEGE OF ARTS, HUMANITIES AND EDUCATION **(CAHE REP)**	☐
COLLEGE OF HEALTH, LIFE AND ENVIRONMENTAL SCIENCES **(CHLES REP)**	☒
Please tick one of the boxes below. *Please consult the relevant guidance on Research Ethics Blackboard page before doing so.*	
FULL REVIEW	☒
PROPORTIONATE REVIEW	☐

SECTION B: RESEARCHER AND PROJECT DETAILS *(Complete relevant sections)*	
Lead Researcher:	Georgie Sherrard
Other Researcher(s):	
Lead Researcher Email: *(Must be a University of Worcester email)*	Sheg21_10@uni.worc.ac.uk
School / Department:	**College of Health, Life and Environmental Sciences**
Status of Lead Researcher:	**MRes student**
Director of Studies *(where applicable**)* ***e.g. for PhD / DBA / MRes students*	Dr Allain Bueno
Project Title:	**Are dietary fatty acids, blood fatty acid composition and anthropometric biomarkers associated with ventricular depolarisation? An observational study in a sample population of healthy pre-menopausal women.**
Is project externally funded or been submitted to an external funder?	No
Name of Funder:	
University of Worcester Funding Bid Reference Number if applicable:	

SECTION C: APPLICATION DOCUMENT CHECKLIST

PLEASE NOTE:

- **All research materials / supporting documentation must be submitted as separate documents with this form.**
- **Please ensure the documents are clearly named to indicate what they are.**
- **Your proposal will not be reviewed without these documents. If these documents are not received by the submission deadline date your proposal will be returned to you.**
- **Please refer to the Research Ethics pages on Blackboard for the most up to date versions of the templates and current guidance documents.**

Please indicate which documents are included:

☒ Participant Information Sheet (PIS) & Privacy Statement *(University of Worcester Template)*
☒ Consent Form *(University of Worcester Template)*
☐ Interview Guide / Schedule
☒ Questionnaires
☐ Letter / Email from Gatekeeper granting access to research site, data or population
☒ Other *(Please specify)* Risk Assessments
➢
☒ Have you included details about how GDPR requirements have been met?
☒ Have you read both the **Research Proposal Checklist Declaration** (Section D) and **Declaration of Researcher / PG Research Student** (Section E)?
☒ Is the application being sent from a University of Worcester email address?
☒ Do you understand that by submitting this form from your University of Worcester email account you are declaring that you have met all of the conditions?
☐ Have you named the College Ethics Panel (e.g. CBPS / CAHE / CHLES) in the subject line of your application email?

PhD / DBA / MRes Students Only

☒ Has your Director of Studies / Supervisor PhD / DBA / MRes had sight of your application form?
☒ Has your Director of Studies / Supervisor been copied into the email sending the application?

SECTION D: RESEARCH PROPOSAL CHECKLIST

		Yes	No
1.	Does your proposed research involve the collection of data from living humans?	☒	☐
2.	Does your proposed research require access to secondary data or documentary material of a sensitive or confidential nature from other organisations?	☐	☒
3.	Does your proposed research involve the use of data or documentary material which (a) is not anonymised **and** (b) is of a sensitive or confidential nature **and** (c) relates to the living or recently deceased?	☐	☒
4.	Does your proposed research involve participants who are particularly vulnerable or unable to give informed consent?	☐	☒
5.	Will your proposed research require the co-operation of a gatekeeper for initial access to the groups or individuals to be recruited?	☐	☒
6.	Will financial inducements be offered to participants in your proposed research beyond reasonable expenses and/or compensation for time?	☐	☒
7.	Will your proposed research involve collection of data relating to sensitive topics?	☐	☒
8.	Will your proposed research involve collection of security-sensitive materials?	☐	☒
9.	Is pain or discomfort likely to result from your proposed research?	☒	☐
10.	Could your proposed research induce psychological stress or anxiety or cause harm or negative consequences beyond the risks encountered in normal life?	☐	☒
11.	Will it be necessary for participants to take part in your proposed research without their knowledge and consent at the time?	☐	☒
12.	Does your proposed research involve deception?	☐	☒
13.	Will your proposed research require the gathering of information about unlawful activity?	☐	☒
14.	Will invasive procedures be part of your proposed research?	☒	☐
15.	Will your proposed research involve prolonged, high intensity or repetitive testing?	☐	☒
16.	Does your proposed research involve the testing or observation of animals?	☐	☒
17.	Does your proposed research involve the significant destruction of invertebrates?	☐	☒
18.	Does your proposed research involve collection of DNA, cells, tissues or other samples from humans or animals?	☒	☐
19.	Does your proposed research involve human remains?	☐	☒
20.	Does your proposed research involve human burial sites?	☐	☒
21.	Will the proposed data collection in part or in whole be undertaken outside the UK?	☐	☒
22.	Does your proposed research involve NHS staff or premises?	☐	☒
23.	Does your proposed research involve NHS patients?	☐	☒

If the answers to any of these questions change during the course of your research, you should seek guidance from the Chair of the relevant Research Ethics Panel.

RESEARCH PROPOSAL CHECKLIST DECLARATION

By submitting this application via my UW email account I am declaring that I have answered the questions above honestly and to the best of my knowledge.

Please note: *The Lead Researcher is, where applicable, submitting on behalf of all researchers involved with the research.*

If you have answered **NO** to all questions you should now submit this form to ethics@worc.ac.uk.

If you have answered **YES** to one or more questions you must now complete **SECTION E** (below) and submit the completed form to ethics@worc.ac.uk identifying the College Research Ethics Panel you wish to review your application in the subject line.

SECTION E: FULL APPLICATION

Details of the Research

Outline the context and rationale for the research, the aims and objectives of the research, and the methods of data collection. This should draw on the previous literature and should be more than simply a set of aims and objectives. The methods of data collection also need to be justified, and the selection of specific measures or tests should be justified in relation to their validity for the population in question.

Coronary heart disease (CHD) is the leading cause of death worldwide, and along with stroke, was responsible for more than half of all deaths globally in 2016, equating to just over 15 million people. These diseases, as well as a host of other conditions, fall under the umbrella of Cardiovascular Disease (CVD). Affecting approximately 7 million people in the UK alone, CVD costs the NHS almost £9 billion a year.

The risk of developing CVD can be increased by a number of different factors, one of which is poor nutrition, meaning that dietary intervention is an important aspect of patient therapy. Fatty acids, in particular the n-3 polyunsaturated group, have been positively implicated with regard to heart health, although the precise mechanisms by which these fatty acids affect things like atherosclerosis, cell proliferation, platelet aggregation and cardiac arrhythmias are not yet fully understood.

This study will establish dietary fatty acids, blood fatty acid composition and a number of anthropometric biomarkers in each of a small population of healthy pre-menopausal women. For each participant, these recordings will then be correlated with the area under the curve (AUC) of the QRS complex of their electrocardiogram (ECG) reading. The AUC of the QRS complex is a reflection of ventricular depolarisation, and therefore indicates heart contractility. Heart contractility is a factor affecting the heart's stroke volume and therefore cardiac output. A thorough literature review will then compare these findings with those reported in previous studies, in order to understand the potential role of the n-3 polyunsaturated fatty acids (PUFAs) in cardiovascular health.

Interested individuals will be given a participant information sheet* (PIS) to give more details about the study and the inclusion criteria. This will be done in person so that they can ask questions if necessary. If they are deemed eligible and decide to take part, then a mutually convenient time will be agreed for the participant to come to the testing laboratory (EEG052). Here they will fill in the participant questionnaire* (for gathering of research data) and sign a consent form* on which they are assigned a participant ID number. They will then undergo a number of measurements and tests, the results from which will be recorded by the Lead Researcher on the measurements/test results section of the participant questionnaire.
The measurements and tests to be carried out are as follows:

- Fatty acid levels in the blood will be quantified from a capillary blood sample, using gas chromatography.
- Haematocrit levels will be measured, using a second capillary blood sample.
- Participants will be subjected to a 4-lead ECG in order to examine ventricular depolarization and to record resting heart rate.
- Participants will be weighed and their heights measured in order that BMI can be calculated.
- Waist and hip measurements will be taken, and waist-hip ratio ascertained.
- Body fat will be measured using bioelectrical impedance.
- A pulse oximeter will be used to determine blood oxygen levels.
- A blood pressure monitor will measure the participant's blood pressure.

These are all important measurements for obtaining a fair representation of an individual's cardiovascular health.

The 4-day food diary* and information on portioning* will then be given to the participant, with an explanation as to how it should be filled in. A stamped addressed envelope will also be provided, so that the food diary can be returned to the Lead Researcher once complete. The food diary will be anonymised, containing only the participant ID number from the corresponding questionnaire and test results.

Data from the above tests will be analysed using Microsoft Excel and IBM SPSS. Scatterplots will be generated to visualise associations between the outcome variable AUC for the QRS segment of the ECG and the following predictors:

n-3 polyunsaturated fatty acid (PUFA) intake (from dietary analysis)
n-6 PUFA intake (from dietary analysis)
total fat (from dietary analysis)
total saturated fat (from dietary analysis)
whole blood n-3 PUFA (from gas chromatography)
whole blood n-6 PUFA (from gas chromatography)
whole blood total PUFA (from gas chromatography)
whole blood total monounsaturated fatty acid (MUFA) (from gas chromatography)

whole blood saturated fatty acid (from gas chromatography)
haematocrit
% body fat
100% - % body fat (non-fat tissue)
body mass index (BMI)
waist:hip ratio
waist circumference
diastolic blood pressure (DBP)
systolic blood pressure (SBP)
mean arterial pressure (MAP)

Pearson product-moment correlation coefficients and significance values (p values) will be calculated for each association. The significance threshold will be set at 0.05. To compare these associations with those reported in previous studies, an integrative literature review will be carried out.

***See attached documents: 'PIS including GDPR privacy notice', 'Questionnaire and test results', 'UW Consent Form (Non-NHS)', 'Study Food Diary', 'Portioning Tips'.**

Who are your participants/subjects? *(if applicable)*

Study participants are required to be female, between the ages of 30-40, with no pre-existing heart conditions. Participants must be pre-menopausal as hormonal changes that take place during menopause have their own effects on the heart. Those who smoke and those who take medications that affect heart rhythm (e.g. beta-blockers) are also excluded.

The study will not consider self-reported family history of CVD or levels of physical activity in participants because it would be introducing new variables (no CVD history versus history, and sedentary versus physically active), which would increase the complexity of the study exponentially. This is a free-living, self-reported healthy sample population.

It is thought that 30 participants is sufficient for this observational study as this sample size is similar to ones in previous published observational studies.

How do you intend to recruit your participants? *(if applicable)*

This should explain the number of participants and the means by which participants in the research will be recruited. If any incentives and/or compensation (financial or other) is to be offered to participants, this should be clearly explained and justified. The sample size should be justified either on the basis of a power analysis, or on the basis of previous studies.

No compensation or financial reward will be offered to participants.
The Lead Researcher will recruit at least 30 participants **by inviting individuals known to fit the criteria** to get involved, as well as advertising around the University for participants*.

***See attached document 'Participant Recruitment'.**

How will you gain informed consent/assent? *(if applicable)*

Where you will provide an information sheet and/or consent form, please append this. The University of Worcester Participation Sheet and Privacy Statement template must be used. If you are undertaking a deception study or covert research, please outline how you will debrief participants below.

A detailed participant information sheet* (PIS) will be given out to all interested individuals prior to the laboratory testing, and participants who report that they fit the criteria for the study and who are willing to take part will be required to fill out a data collection questionnaire* and sign a consent form* at the point of laboratory testing. Participants will have the chance to ask questions at these points.

***See attached documents 'PIS including GDPR privacy notice', 'Questionnaire and test results' 'UW Consent Form (Non-NHS)'.**

Confidentiality, Anonymity, Data Storage and Disposal *(if applicable)*

Provide explanation of any measures to preserve confidentiality and anonymity of data, including specific explanation of data storage and disposal plans. Plans for data storage and disposal must be feasible given the nature of the study.

Participant names will only be recorded on consent forms*. A participant ID number will be assigned to each participant on the consent form, with this number then being used on all further paperwork and computer files. Consent forms and anonymised food diaries, participant questionnaires and test result sheets will be collected by **the Lead Researcher** only**. Consent forms will be kept separately from other hard-copy forms to ensure anonymity is preserved. They will all be securely stored in dedicated folders in a locked cabinet** in the **Study**

Supervisor's office **(Dr Allain Bueno – EE!002)** in accordance with the University of Worcester Ethics Policy, the University of Worcester Information Security Policy, and the General Data Protection Regulations (GDPR) 2018. **This office is locked when empty.**

All information gathered from individuals through questionnaires and test results that is subsequently transferred to documents on the Lead Researcher's personal computer will include only participant ID numbers, and therefore individuals will not be identifiable from this information. These documents will also be kept in a password-protected folder on the computer's hard disk. The computer itself requires a password in order to use it.

All personal data, whether hard-copy or on computer, will be destroyed within three months after the end of the study. It is hoped that the study will be finished by September 2020.

Blood samples will be dealt with in accordance with the Human Tissue Act 2004. The sample taken for measuring haematocrit will be processed immediately and then destroyed. The sample taken for fatty acid analysis will be immediately treated with butylated hydroxytoluene (BHT), which is an antioxidant that denatures DNA, RNA and protein. The cells in the sample will therefore be rendered acellular, and can then be labelled with the participant's ID number and stored securely in a University -80^0 freezer in a locked laboratory (EEG070) until they are processed. Once processed, samples will be destroyed.

***See attached documents 'UW Consent Form (Non-NHS)'.**

Potential Risks to Participants / Subjects / Researcher *(if applicable)*

Identify any risks for participants/subjects that may arise from the research and how you intend to mitigate these risks. Potential risks to the researcher must also be considered. Risks may include physical, practical, psychological and emotional consequences of participation.

The risks associated with the laboratory tests are no greater than those expected in normal life. In saying that, precautions will always be taken, including the use of a lab coat and gloves. Participants will also be asked to wash their hands, in order to minimise the chances of minor infection from the finger prick test. A separate lancet will be used for each individual and discarded in the sharps bin after use. **The bioelectrical impedance monitoring and the ECG pose no risk above the possibility of someone being allergic to the methacrylates on the ECG electrodes. This is rare, and usually occurs with prolonged adhesion, however, a warning is included on the PIS* document in the inclusion criteria section, and this allergy is asked about on the participant questionnaire* also.**

The Lead Researcher will receive comprehensive instruction on the procedures to be used and the safe use of all laboratory equipment before any participants are seen. A third party will always be present in the laboratory to ensure help can be quickly sought if necessary.

Ethical considerations include the collection of data pertaining to BMI and body fat percentage, which some may consider sensitive. **After consent is obtained, all forms pertaining to an individual participant are assigned a participant ID number and kept separately from consent forms*, so that this information can be kept anonymous. The Lead Researcher and the Study Supervisor** are the only two people to deal with personal information and all forms will be kept in dedicated confidential files in a **locked cabinet** in the **Study Supervisor's** office (EE1002) **which is locked when not in use.**

Thorough risk assessments* have been carried out, and these will be kept with the Lead Researcher at all times when in the lab.

***See attached documents: 'PIS including GDPR privacy notice', 'Questionnaire and test results', 'UW Consent Form (Non-NHS)' 'Fatty acid analysis prep – lab risk assessment' and 'Risk assessment for participant tests'.**

Other Ethical Issues

Identify any other ethical issues (not addressed in the sections above) that may arise from your research and how you intend to address them.

Participants might want further information about what their test results could mean, so it will be made clear to them on the information sheet* that the Lead Researcher is not medically trained and is therefore unable to give medical advice. This is reiterated on the consent form*. It is also stated that the ECG is not a diagnostic tool and therefore has no clinical validity. Participants will be advised to speak to a healthcare provider if they have any concerns following the laboratory testing.

See attached documents 'PIS including GDPR privacy notice', 'UW Consent Form (Non-NHS)'.

Published Ethical Guidelines to be followed

Identify the professional code(s) of practice and/or ethical guidelines relevant to the subject of the research.

University of Worcester Ethics Policy
University of Worcester Information Security Policy
General Data Protection Regulations (GDPR) and UK Data Protection Bill (2018)

DECLARATION OF RESEARCHER / PG RESEARCH STUDENT

By submitting this form via your University of Worcester email account, you are confirming the following:

- I have read the University Ethics Policy and any relevant codes of practice or guidelines and I have identified and addressed the ethical issues in my research honestly and to the best of my knowledge and by submitting this form to ethics@worc.ac.uk.

- I confirm that I have a research data management plan in place in accordance with the policy for the effective management of research data.

Institute of Science and the Environment

LABORATORY RISK ASSESSMENT FORM

Name	**Georgie Sherrard**	**Please seek help if you have difficulty filling out this form. There are guidelines on Blackboard, see your supervisor or a Technician.** RISK LEVEL RANKING (Low, Medium, High, Urgent) If any are high or urgent please seek advice. See guidance for how to calculate the risk.
Type of Lab Work e.g. independent student project, research	**Masters research**	
Dates: From - To	**1st March 2019 – 31st March 2020**	
Location(s) e.g. EE1043	**EEG052** **EE1040 - 1041**	

Potential hazardous procedures

- Performing and recording electrocardiography
- Bioelectrical impedance monitoring
- Blood pressure monitoring
- Finger prick capillary blood collection
- Use of centrifuge and other electrical devices

HAZARDS	WHO is at RISK?	DESCRIPTION of INJURY or ILLNESS	CONTROL MEASURES	RISK LEVEL RANKING (Low, Medium, High, Urgent)
Physical hazards Broken glassware	Untrained person Glassware handler Project participant	Cuts from damaged or broken glass (capillary blood collection tubes)	• Check all glassware before use • Glass transported carefully • Never store glassware on floor • Glass "sharps" must be disposed of in the proper containers and not in the ordinary waste-bins • If cut, call a first aider (List next to phone and in ISE Health and Safety booklet)	Low
Lancet needles	Untrained person Project participant	Risk of infection Risk of bruising at test site Risk of fainting due to the sight of blood	• Participant to wash hands thoroughly before finger prick • Wipe site area with an alcohol wipe prior to blood collection • Cover test site with a sterile plaster • Researcher to wear safety gloves during procedure • Fingers must not be squeezed excessively to draw blood. If insufficient blood is collected, warm the hands and try again with a different finger • Used lancets must be disposed of in the proper container • Participant to sit down while blood is collected	Medium

Bioelectrical impedance monitoring	Project participant	Risk to those who have heart disease or a pacemaker	• Explain hazards in full to each participant • Eliminate any participants who are at risk	Low
Performing and recording electrocardiography	Untrained person Project participant	Muscular strain and pain on neck and lower back from static and/or awkward postures Serious or fatal Injury from electrical shock/ burn due to contact with live electrical parts	• Brief participants on ergonomic risk factors and the need to be comfortable while remaining still • Use a mat and pillow underneath the participant • Training on proper use of equipment and safe work practices e.g. avoid touching wiring with wet hands • Report any discrepancies such as visible damage to the machine to supervisor for corrective actions • Ensure periodic inspection and maintenance of the equipment by a qualified person such as an approved technician	Medium
Blood pressure monitoring	Project participant	Discomfort Potential for ulnar nerve palsy and venous haemostasis	• Training on proper use of equipment and correct cuff placement	Medium
Electrical devices e.g. computer, centrifuge, pulse oximeter	Researcher Project participant	Electrical shock Electrical burns Electrical fires Exposure to infra-red light from pulse oximeter	• Training on proper use of equipment if necessary • Ensure equipment is used in a safe environment at all times i.e. clean and dry • Follow manufacturers' instructions, including regular checks on electrical fittings and electrical apparatus • Use P.P.E. (Personal Protective Equipment) if necessary	Medium

Risk of fainting	Project participant	Potential reaction to the sight of one's own blood	• Ensure participants are made aware of the risk on the participant questionnaire • Risk assessed with participant before procedure occurs • All participants are to be seated for the procedures where blood is taken • Have a trained first aider present if seeing participants in the lab over a weekend.	Medium
Biological hazards (e.g. body fluids, blood samples)	Untrained person Sample handler	Infection (bodily fluids, respiratory, skin contact)	• Use P.P.E. (Personal Protective Equipment) – Lab coat, gloves, eye protection	High
Allergies	Project participant	Allergy to any of the following: Latex, plasters, rubber, methacrylates	• Ensure participants are aware of the potential allergens and have notified Lead Researcher if an allergy is known • Do not include participants if a known allergy exists • Seek immediate medical attention if an allergic reaction occurs	Low
Chemical hazards (e.g. irritants; toxins; carcinogens; vapours; dusts; contaminated soils) See COSHH forms for specific hazards.	Untrained person Chemical handler Persons working in vicinity	Chemical burns Incapacitation due to fumes Respiratory damage due to inhalation Allergic reaction Asphyxiation	• Follow COSHH forms when handling chemicals following personal precautions, protective equipment, emergency procedures and disposal • Be aware of hazards arising from substances or mixture – can become explosive, be carcinogenic etc • Use P.P.E (Personal Protective Equipment) – Lab coat, gloves, face and respiratory protection • Use fume hoods where necessary • Store in appropriate manner – lockable, cool dry environment • Be aware of methods and material for containment	High

		Poisoning from ingestion	and cleaning up – e.g. inert absorbent material, disposal routes, closed containers for disposal **Emergency procedures:** • **General advice:** Consult a physician. Show the safety data sheet to the doctor in attendance. • **If inhaled:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician or emergency services • **In case of skin contact:** Wash as per COSHH form. Take victim immediately to hospital if necessary. Consult a physician. In case of eye contact rinse thoroughly as per COSHH form for at least 15 minutes and consult a physician. • **If swallowed:** Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.	
Personal safety (e.g. lone working, other workers, illness, disability)	Persons on premises	Lack of help and support in case of emergency	• Avoid lone working where possible. • Have a trained first aider present if seeing participants in the lab over a weekend. • Inform another member of the university you are on site (e.g. Security) where they can provide regular checks	Medium
Environmental impact (e.g. waste; pollution)	Environmental habitats	Disrupt environmental habitat affecting wildlife and plant-life	• Prevent environmental leakage, do not let chemicals enter drains	Low

Other hazards (e.g. manual handling)	Untrained person	Injury due to poor manual handling techniques (e.g. twisting, stooping, stretching, excessive lifting, lowering or carrying distances, strenuous pushing or pulling)	• Correct manual handling procedures adhered to – complete training where necessary	Low

	(tick boxes)					
Are harmful substances used? (if yes a COSHH form must be completed and attached)	**YES**	x	**NO**		**N/A**	
Necessary training and information received?	**YES**	x	**NO**		**N/A**	
Other?	**YES**		**NO**		**N/A**	

It can be difficult to foresee all risks and hazards that might be associated with the work, when arriving at place of work any additional hazards should be assessed at that time and appropriate action taken and recorded.

Unforeseen hazards may arise during the work, always put health and safety first, do not take risks, if in doubt stop work

Person completing this assessment:

Name .....Georgie Sherrard..... Signature..... Date……14/3/2019……

Name ..... Signature..... Date……..

Independent Study/ Postgraduate Supervisor:

Name ..... Signature ..... Date……..

Institute of Science and the Environment

LABORATORY RISK ASSESSMENT FORM

Name	**Georgie Sherrard**	**Please seek help if you have difficulty filling out this form. There are guidelines on Blackboard, see your supervisor or a Technician.** **RISK LEVEL RANKING (Low, Medium, High, Urgent) If any are high or urgent please seek advice. See guidance for how to calculate the risk.**
Type of Lab Work e.g. independent student project, research	**Masters research**	
Dates: From - To	**1st March 2019 – 1st September 2019**	
Location(s) e.g. EE1043	**EE1040 – 1041** **EEG052**	

FATTY ACID ANALYSIS

Extraction method

Process tissue (blood, plasma, cells) samples with Methanol/Chloroform/BHT (C/M/BHT) in Borosilicate glass test tubes with socket quickfit. With caps on, flush the samples with oxygen-free nitrogen (OFN) for 1 minute. Store samples for 24hours at 4ºC

Partitioning

Filtered the samples using Whatman filter paper, with vessels washed with Chloroform/Methanol/BHT. Add 0.85% Saline to filtered sample. With caps on, flush the samples with OFN for 1 minute. Store samples overnight at 4ºC.

Rotary Evaporation

Samples will be brought to room temperature over 30 minutes. Drain the lower organic layer of the sample into Borosilicate glass round bottom flask. Using rotary evaporator, remove solvent under reduced pressure in water bath at 37ºC. When dry add 1-2ml methanol, rinse flask, then rotavap again to dry. Repeat twice to remove any residual water. Remove dried lipid extract to 10ml borosilicate glass test tube with Teflon liner cap using 3 X 2ml washes of C/M/BHT. Clean glass connector 3 times with C/M before next sample. Reduce to a volume of 1 ml under stream of OFN for 1 minute. Store at 4ºC or -20ºC until needed.

Methylation

Prepare methylating reagent

Wearing a face mask and fully buttoned lab coat, dropwise add 15ml acetyl chloride to 100ml dry methanol in 500ml conical flask while swirling the flask under a cold stream of cold water or over ice, being careful not to allow any water splashes to enter vessel, nor allowing the acetyl chloride to boil in the methanol. Transfer mixture to stoppered bottle.

To the stored samples add 4ml of methylating reagent using a 4 X 1ml Pasteur pipette. With the cap held over the neck of the sample, flush the sample thoroughly with OFN through the liquid. Secure the cap tightly and vortex sample. Mark level of liquid in the tube with a marker pen. Methylate at 70ºC for three hours in an oven. At both 1 and 2 hours check level of sample with marked line, and if decreased, top up with methanol, re-flush and change the Teflon lined cap and/or tube. Vortex the tube before replacing in the oven.

Extraction of methyl esters

Remove the tube from the oven and allow to come to room temperature.

For methyl esters: To each tube add 4ml 5% Saline solution/distilled water and 2ml petrol spirit +BHT. Cap and shake well.
For Propyl esters: To each tube add 2ml 5% Saline solution/distilled water and 2ml petrol spirit +BHT. Cap and shake well.

If emulsion has formed break it with a few drops of methanol, or centrifuge tube on very short slow spin. Remove the upper petrol layer to a test-tube containing 2ml 2% potassium bicarbonate. Add 1ml petrol to methylating tube, mix, spate and pool petrol extract. Add 1 ml again and pool, making a total extract of 2+1+1=4ml. Vortex petrol/potassium bicarbonate sample and transfer the upper, petrol layer to a test tube containing 100-200mg dried granular sodium sulphate to remove residual water. Remove the solution of fatty acid methyl esters in petrol to a 3ml vial avoiding transferring any sodium sulphate granules. Remove petrol under stream of OFN. Take up sample in 1ml heptane +BHT if the sample does not need cleaning up, or 1 ml petrol if it does. Flush with OFN and store at 4ºC or -20ºC until ready for cleaning up or GLC.

HAZARDS	WHO is at RISK?	DESCRIPTION of INJURY or ILLNESS	CONTROL MEASURES	RISK LEVEL RANKING (Low, Medium, High, Urgent)
Physical hazards Broken glassware	Untrained person Glassware handler	Cuts from damaged or broken glass. Cuts from flying glass due to implosion following evacuation or mechanical shock or stress. Cuts from forcing plastic tubing, teats or rubber bungs onto glass tubing, pipettes or condensers that break. Cuts from broken glass and sharp items e.g. Pasteur pipettes disposed in ordinary wastebins. Burns from heated glass. Poisoning following cuts by contaminated glassware.	• Check all glassware before use. • Glass transported carefully • Never store glassware on floor • When fitting tubing to glassware, glass may be lubricated with water or glycerol and the plastic tubing softened by brief immersion in hot water. Excessive force must not be used or force in a direction which will make the glass snap. Thought should be given as to where the sharp edge of the glass might go if it does break and the grip arranged accordingly. The glass may be wrapped in a towel or thick layers of paper tissue. When tubing is being removed, a sharp knife can be used to cut off tubing that does not yield to gentle pressure. Hot glass (which looks the same as cool glass) should be treated with care and placed where no one can accidentally come into contact with it before it has cooled. • Joints and stoppers- Ground glass connections should be lubricated before assembling and	High

			disassembled immediately after use. Flasks or containers must not be stoppered when hot. If a stopper seizes, it is extremely dangerous to reheat the container to remove it. • Damaged glassware should be repaired or disposed of in the "Broken Glass" bin and not the ordinary waste-bins. A brush and dustpan should be used to clear up broken glass. Special care is needed when clearing broken glass from a sink where water can make sharp edges invisible: tongs can be used to pick out pieces. • Glass "sharps" must be disposed of in the proper containers and not in the ordinary waste-bins. • Broken glass must be disposed of into specially designated bins and not into the normal waste bins. Use dustpan and brush. • If cut, call a first aider (List next to phone and in ISE Health and Safety booklet) • Use P.P.E (Personal Protective Equipment): If pressure is needed to fit tubing, use leather gloves covering the wrists or towel/tissue/padding as required	

Fire, explosion	Untrained person Chemical handler Persons challenged (physically, visually, mentally etc) Persons unable to react quickly	Burns Smoke inhalation Asphyxiation Physical harm from flying debris	• Fire Procedure and Training for persons at risk • Know location of emergency exits, fire alarms, fire extinguishers, sand, first aid kit, first aiders. Ensure that fire evacuation signs and fire routines are satisfactory. • Follow manufacturer's instructions, including regular checks on electrical fittings and electrical apparatus. • Allow heated areas such as ovens, inlet, detectors etc to cool down before touching them • Periodic visual inspections and pressure leak test on sampling equipment and gas cylinder and supply systems. • Flammable chemicals kept away from sources of flame (e.g. Matches, lighters, candles, smoking materials, Bunsen burners, ovens) and used in fume cupboard where necessary • Correct use of combustibles including correct handling of chemicals as per individual COSHH forms • Keep work area clean and tidy, devoid of additional combustible material (paper, polystyrene etc). • Use P.P.E (Personal Protective Equipment)	Medium

Electrical shock	Untrained person Chemical handler	Electrical shock Electrical burns	• Turn off the instrument and disconnect the power cord at its receptacle whenever accessing power inlets on electrical equipment • Keep areas of electrical input clean and dry • Follow manufacturer's instructions, including regular checks on electrical fittings and electrical apparatus. • Use P.P.E (Personal Protective Equipment)	Medium
Biological hazards (e.g. micro-organisms; animal tissue; body fluids; plant material) use your COSHH form to assist here.	Untrained person Tissue handler	Infection (bodily fluids, respiratory, skin contact) Allergic reaction Poisoning from injection	• Use P.P.E (Personal Protective Equipment) – Lab coat, gloves, eye protection • Follow BioCOSHH risk assessment for biological agents and hazards for correct handling and storage procedures • Regular monitoring by PI/technician to ensure controls are effective and complied with • Regularly review BioCOSHH risk assessment and amend where necessary if any changes in activity occur.	High

Chemical hazards (e.g. irritants; toxins; carcinogens; vapours; dusts; contaminated soils) Use your COSHH form to assist here.	Untrained person Chemical handler Persons working in vicinity	Chemical burns Incapacitation due to fumes Respiratory damage due to inhalation Allergic reaction Asphyxiation Poisoning from ingestion	• Follow COSHH forms when handling chemicals following personal precautions, protective equipment, emergency procedures and disposal • Be aware of hazards arising from substances or mixture – can become explosive, be carcinogenic etc • Use P.P.E (Personal Protective Equipment) – Lab coat, gloves, face and respiratory protection • Use fume hoods where necessary • Store in appropriate manner – lockable, cool dry environment • Be aware of methods and material for containment and cleaning up – e.g. inert absorbent material, disposal routes, closed containers for disposal **Emergency procedures:** • **General advice:** Consult a physician. Show the safety data sheet to the doctor in attendance. • **If inhaled:** If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician or emergency services • **In case of skin contact:** Wash as per COSHH form. Take victim immediately to hospital if necessary.	High

			Consult a physician. In case of eye contact rinse thoroughly as per COSHH form for at least 15 minutes and consult a physician. • **If swallowed:** Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.	
Man-made hazards (e.g. electrical equipment; uv light)	Untrained person	Electrical shock Electrical burns Electrical fires Explosions	• Turn off the instrument and disconnect the power cord at its receptacle whenever accessing power inlets on electrical equipment • Keep areas of electrical input clean and dry • Follow manufacturer's instructions, including regular checks on electrical fittings and electrical apparatus. • Use P.P.E (Personal Protective Equipment) where necessary	Medium

Personal safety (e.g. lone working, other workers, illness, disability)	Persons on premises	Lack of help and support in case of emergency	Avoid lone working where possible Inform another member of the university you are on site (e.g. Security) where they can provide regular checks	Medium
Environmental impact (e.g. waste; pollution)	Environmental habitats	Disrupt environmental habitat effecting wildlife and plantlife	• Prevent environmental leakage, do not let chemicals enter drains	Low

Other hazards (e.g. manual handling)	Untrained person	Injury due to poor manual handling techniques (e.g. twisting, stooping, stretching, excessive lifting, lowering or carrying distances, strenuous pushing or pulling)	Correct manual handling procedures adhered to – complete training where necessary	Low

Are harmful substances used? (if yes a COSHH form must be completed and attached)	**YES**	X	**NO**		**N/A**	
Necessary training and information received?	**YES**	X	**NO**		**N/A**	
Other?	**YES**		**NO**		**N/A**	

It can be difficult to foresee all risks and hazards that might be associated with the work, when arriving at place of work any additional hazards should be assessed at that time and appropriate action taken and recorded.

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Person completing this assessment:

Name .....Georgie Sherrard..... Signature..... Date……13/2/2019……

Name ..... Signature..... Date……..

University of Worcester	**Institute of Science and the Environment** **Control Of Substances Hazardous to Health (COSHH) ASSESSMENT FORM**

Name of chemical	Chloroform	CAS-no: 67-66-3	EC No: 200-663-8

Will the chemical be used in its **undiluted**/solid state (underline/circle as appropriate):

YES	NO

Appearance	Liquid	**Initial boiling point and range**	62°C @ 760 mmHg
Colour	Colourless	**Melting point**	-63.5°C
Odour	Pleasant, agreeable, sweetish	**Relative density**	1.49 g/cm3 @ 20°C
Solubility	Slightly soluble in water	**Vapour pressure**	245 mm Hg @ 30°C

1. Hazard Symbol(s):

2. Risk Phrase(s) or Hazard Statement(s):

H30	Harmful if swallowed or if inhaled
H31	Causes skin irritation
H31	Causes serious eye irritation.
H33	May cause drowsiness or dizziness.
H351	Suspected of causing cancer
H361	Suspected of damaging the unborn child.
H373	May cause damage to organs through prolonged or repeated

3. Safety Phrase(s) or Precautionary Statement(s):

P281	Use personal protective equipment as required.
P20	Do not handle until all safety precautions have been read and understood.
P260	Do not breathe dust/fume/gas/mist/vapours/spray
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P302+352	IF ON SKIN: Wash with plenty of soap and water.
P314	Get medical advice/attention if you feel unwell.
P501	Dispose of contents / container to hazardous waste depot
P281	Use personal protective equipment as required.
P405	Store locked up
P301	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell.
P330	Rinse mouth.

Personal precautions, protective equipment, emergency procedures and disposal

Gloves, lab coat and safety glasses to be worn at all times
Exposure route: Inhalation, Ingestion; contact with skin and eyes.
Use chemical fume cupboard when handling neat solution.

Accidental release measures: Wear respiratory protection. Avoid breathing vapours, mist or gas. Ensure adequate ventilation. Evacuate personnel to safe areas.
Methods and materials for containment and cleaning up: Soak up with inert absorbent material and dispose of as hazardous waste. Keep in suitable, closed containers for disposal.
Environmental precautions: Prevent further leakage or spillage if safe to do so. Do not let product enter drains. Discharge into the environment must be avoided.
Hazards arising from substance or mixture: Can decompose to form hydrogen chloride and phosgene. May form explosive mixtures with oxidising agents and metals.

Emergency procedures:
General advice: Consult a physician. Show this safety data sheet to the doctor in attendance.
If inhaled: If breathed in, move person into fresh air. If not breathing, give artificial respiration. Consult a physician.
In case of skin contact: Wash off with soap and plenty of water. Take victim immediately to hospital. Consult a physician. In case of eye contact Rinse thoroughly with plenty of water for at least 15 minutes and consult a physician.
If swallowed: Never give anything by mouth to an unconscious person. Rinse mouth with water. Consult a physician.
Firefighting measures: Use water spray (NOT JET), alcohol-resistant foam, dry chemical or carbon dioxide. Do not use carbon dioxide in enclosed spaces with insufficient ventilation.
Special hazards arising from the substance or mixture: Can decompose to form hydrogen chloride and phosgene. May form explosive mixtures with oxidising agents and metals. Storage containers can pressurise and explode in the heat of a fire. In case of fire, irritating, toxic or corrosive vapours or mists may be produced. Hydrogen chloride (HCl). Phosgene ($COCl_2$).
Advice for Fire fighters: Wear self-contained breathing apparatus where possible.

The work will be carried out (tick one or more)	
On an open bench	
In a fume cupboard	✓
In a laminar flow cupboard	
Behind a shield	
In a containment cabinet	
Other (specify)	

Personal Protective Equipment Required (tick one or more)	
Eye	✓
Hand	✓
Face	✓
Respiratory	✓
Foot	
Other (specify) Lab coat	✓

How will the chemical be disposed?

Dispose of small quantity of chloroform by evaporation in a chemical fume hood.

Large quantity must be disposed of in waste collection drum located in outside chemical store room (EEG SO16). Contact Noel Egginton (Chemistry/Environmental Technician). Institute of Science & the Environment. Contact Details. email: n.egginton@worc.ac.uk. tel: 01905 855210

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Acetyl Chloride**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?	**15% of acetyl chloride in methanol**

1. Hazard Symbol(s):

2. Risk Phrase(s) or Hazard Statement(s):

H225	Highly flammable liquid and vapour
H302	Harmful if swallowed
H314	Causes severe skin burns and eye damage

3. Safety Phrase(s) or Precautionary Statement(s):

P210	Keep away from heat/sparks/open flames/hot surfaces. - No smoking
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection
P305+P351+P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310	Immediately call a POISON CENTER or doctor/physician.
	Reacts violently with water.

4. Personal precautions, protective equipment, emergency procedures and disposal

Use personal protective equipment. Evacuate personnel to safe areas. Remove all sources of ignition. Take precautionary measures against static discharges. Do not get in eyes, on skin, or on clothing.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench		Eye	X
In a fume cupboard	X	Hand	X
In a laminar flow cupboard		Face	X
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify) - protective	X

How will the chemical be disposed?

Avoid release to the environment.
Reacts with water so no ecotoxicity data for the substance is available.

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**BHT; Butylated hydroxytoluene**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?	

1. Hazard Symbol(s):

				

2. Risk Phrase(s) or Hazard Statement(s):

H411	Toxic to aquatic life with long lasting effects

3. Safety Phrase(s) or Precautionary Statement(s):

P273	Avoid release to the environment

4. Personal precautions, protective equipment, emergency procedures and disposal

Avoid inhalation of vapours/aerosols or dusts. Keep away from heat and sources of ignition. Evacuate the danger area, observe emergency procedures, consult an expert.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench	x	Eye	
In a fume cupboard		Hand	X
In a laminar flow cupboard		Face	X
Behind a shield		Respiratory	
In a containment cabinet		Foot	
Other (specify)		Other-Flame retardant antistatic protective clothing.	X

Do not allow product to reach sewage system or open water

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Heptane**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?

1. **Hazard Symbol(s):**

2. **Risk Phrase(s) or Hazard Statement(s):**

H225	Highly Flammable liquid and vapour
H304	May be fatal if swallowed and enters airways
H315	Causes skin irritation
H336	May cause drowsiness or dizziness
H410	Very toxic to aquatic life with long lasting effects

3. **Safety Phrase(s) or Precautionary Statement(s):**

P210	Keep away from heat/sparks/open flames/hot surfaces. — No smoking.
P301 + P310	IF SWALLOWED+ Immediately call a POISON CENTER or doctor/physician.
P331	Do NOT induce vomiting.
P370 + P378	In case of fire: Use CO_2 for extinction
P403 + P235	Store in a well-ventilated place. Keep cool.

4. **Personal precautions, protective equipment, emergency procedures and disposal**

Use personal protective equipment. Remove all sources of ignition. Take precautionary measures against static discharges. Avoid contact with skin, eyes and clothing. Ensure adequate ventilation.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench		Eye	X
In a fume cupboard	X	Hand	X
In a laminar flow cupboard		Face	
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify)	

5. How will the chemical be disposed?

Do not flush into surface water or sanitary sewer system. Do not allow material to contaminate ground water system. Prevent product from entering drains. Local authorities should be advised if significant spillages cannot be contained. Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment. The product contains following substances which are hazardous for the environment.

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Methanol**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?	

1. Hazard Symbol(s):

2. Risk Phrase(s) or Hazard Statement(s):

H225	Highly flammable liquid and vapour
H301	Toxic if swallowed
H311	Toxic in contact with skin
H331	Toxic if inhaled
H370	Causes damage to organs

3. Safety Phrase(s) or Precautionary Statement(s):

P210	Keep away from heat/sparks/open flames/hot surfaces. - No smoking
P280	Wear protective gloves/ protective clothing/ eye protection/ face protection
P301+ P310	IF SWALLOWED: Immediately call a POISON CENTER or doctor/ physician
P302 + P350	IF ON SKIN: Gently wash with plenty of soap and water
P304 + P340	IF INHALED: Remove to fresh air and keep at rest in a position comfortable for breathing
P240	Ground/Bond container and receiving equipment

4. Personal precautions, protective equipment, emergency procedures and disposal

Wear personal protective equipment. Do not breathe vapours or spray mist. Do not get in eyes, on skin, or on clothing. Use only under a chemical fume hood. Do not ingest. Keep away from open flames, hot surfaces and sources of ignition. Use only non-sparking tools. To avoid ignition of vapours by static electricity discharge, all metal parts of the equipment must be grounded. Take precautionary measures against static discharges.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench		Eye	X
In a fume cupboard	X	Hand	X
In a laminar flow cupboard		Face	X
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify) - protective	X

How will the chemical be disposed?	Waste is classified as hazardous
Do not dispose of waste into sewer. Can be incinerated, when in compliance with local regulations.	
Dispose of this container to hazardous or special waste collection point.	

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	Petroleum ether, boiling range 40-60°C

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?

1. Hazard Symbol(s):

2. Risk Phrase(s) or Hazard Statement(s):

H224	Extremely flammable liquid and vapour
H304	May be fatal if swallowed and enters airways
H336	May cause drowsiness or dizziness
H411	Toxic to aquatic life with long lasting effects

3. Safety Phrase(s) or Precautionary Statement(s):

P210	Keep away from heat/sparks/open flames/hot surfaces. — No smoking
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P273	Avoid release to the environment.
P301 + P310	IF SWALLOWED+ Immediately call a POISON CENTER or doctor/physician
P331	Do NOT induce vomiting.

4. Personal precautions, protective equipment, emergency procedures and disposal

Use personal protective equipment. Ensure adequate ventilation. Keep people away from and upwind of spill/leak. Evacuate personnel to safe areas. Remove all sources of ignition. Take precautionary measures against static discharges

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench	X	Eye	X
In a fume cupboard	X	Hand	X
In a laminar flow cupboard		Face	X
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify)	

5. How will the chemical be disposed?

Should not be released into the environment.

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Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Potassium bicarbonate**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?	**2% of potassium bicarbonate in distilled water**

1. **Hazard Symbol(s):**

2. **Risk Phrase(s) or Hazard Statement(s):**

H320	Causes eye irritation
H316	Causes mild skin irritation
H332	Harmful if inhaled
H335	May cause respiratory irritation

3. **Safety Phrase(s) or Precautionary Statement(s):**

P261	Avoid breathing dust, fume, gas, mist, vapours, or spray
P264	Wash hands thoroughly after handling
P271	Use only outdoors or in a well-ventilated area

4. **Personal precautions, protective equipment, emergency procedures and disposal**

Avoid breathing dust. Avoid contact with skin and eyes. Wash thoroughly after handling. Do not eat, drink, or smoke when using this product. Wear appropriate personal protective equipment recommended.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench	X	Eye	X
In a fume cupboard		Hand	X
In a laminar flow cupboard		Face	
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify)	

How will the chemical be disposed?

Reuse or reprocess, if possible

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Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Sodium Chloride**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used? **5% of sodium chloride in distilled water**

1. **Hazard Symbol(s):**

2. **Risk Phrase(s) or Hazard Statement(s):**

3. **Safety Phrase(s) or Precautionary Statement(s):**

4. **Personal precautions, protective equipment, emergency procedures and disposal**

Wash immediately with soap and water

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench	X	Eye	X
In a fume cupboard		Hand	X
In a laminar flow cupboard		Face	
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify)	

5. **How will the chemical be disposed?**

Not regarded as dangerous for the environment.

University of Worcester

Institute of Science and the Environment
Control Of Substances Hazardous to Health
(COSHH) ASSESSMENT FORM

Name of chemical	**Sodium sulphate**

Will the chemical be used in its undiluted/solid state (underline/circle as appropriate):	YES	NO

If no, how will it be used?

1. Hazard Symbol(s):

2. Risk Phrase(s) or Hazard Statement(s):

H320	Causes eye irritation
H335	May cause irritation to respiratory tract.
H315	May cause irritation to skin.

3. Safety Phrase(s) or Precautionary Statement(s):

4. Personal precautions, protective equipment, emergency procedures and disposal

Do not ingest. Do not breathe dust. Avoid contact with eyes. Wear suitable protective clothing. If ingested, seek medical advice immediately and show the container or the label. Keep away from incompatibles such as oxidizing agents, metals.

The work will be carried out (tick one or more)		Personal Protective Equipment Required (tick one or more)	
On an open bench	X	Eye	X
In a fume cupboard		Hand	X
In a laminar flow cupboard		Face	
Behind a shield		Respiratory	X
In a containment cabinet		Foot	
Other (specify)		Other (specify)	

5. How will the chemical be disposed?

The product itself and its products of degradation are not toxic.

PARTICIPANT INFORMATION SHEET AND PRIVACY NOTICE

TITLE OF PROJECT:
Are dietary fatty acids, blood fatty acid composition and anthropometric biomarkers associated with ventricular depolarisation? An observational study in a sample population of healthy pre-menopausal women.

Invitation

The University of Worcester engages in a wide range of research, which seeks to provide greater understanding of the world around us, to contribute to improved human health and well-being and to provide answers to social, economic and environmental problems.

We would like to invite you to take part in one of our research projects. Before you decide whether to take part, it is important that you understand why the research is being done, what it will involve for you, what information we will ask from you, and what we will do with that information.

We will in the course of this project be collecting personal information. Under General Data Protection Regulation 2016, we are required to provide a justification (what is called a "legal basis") in order to collect such information. The legal basis for this project is "**task carried out in the public interest**".

You can find out more about our approach to dealing with your personal information at https://www.worcester.ac.uk/informationassurance/visitor-privacy-notice.html.

Please take time to read this document carefully. Feel free to ask the researcher any questions you may have and to talk to others about it if you wish. You will have at least 14 days to decide if you want to take part.

What is the purpose of the research?

CVD affects approximately 7 million people in the UK alone and costs the NHS almost £9 billion a year. The risk of developing CVD can be increased by a number of different factors, one of which is poor nutrition, meaning that dietary intervention is an important aspect of patient therapy. Fatty acids, in particular the n-3 polyunsaturated group, have been positively implicated with regard to heart health and this study will investigate whether there are any correlations between dietary fatty acid intake and cardiovascular biomarkers.

Who is undertaking the research?

Georgie Sherrard
MRes Biology student
Sheg21_10@uni.worc.ac.uk

Who has oversight of the research?

The research has been approved by the Research Ethics Panel for the College of Health, Life and Environmental Sciences in line with the University's Research Ethics Policy. The University of Worcester acts as the "Data Controller" for personal data collected through its research projects & is subject to the General Data Protection Regulation 2016. We are registered with the Information Commissioner's Office and our Data Protection Officer is Helen Johnstone (infoassurance@worc.ac.uk). For more on our approach to Information Assurance and Security visit: https://www.worcester.ac.uk/informationassurance/index.html.

What are the inclusion criteria?

You must be a non-smoking female, between the ages of 30 and 40, who is pre-menopausal. You must not have a pre-existing heart condition or type 2 diabetes, and no diagnosis of lung, kidney or liver disease. Those with a pacemaker or a diagnosis of a psychiatric disorder are not eligible. You must not be taking blood-thinners, beta-blockers or any other medication that can affect heart rhythm.

Please note: the adhesive on the electrocardiogram (ECG) electrodes contains methacrylates, which are also found in acrylic nails, paint, varnish, printing ink, adhesives, glue, orthopaedic prostheses, bone cement and dental restorative materials. If you are known to have an allergy to any of these things, you will not be allowed to take part.

Why have I been invited to take part?

You have received this invitation because it is possible that you meet the criteria for inclusion (see above). We are hoping to recruit 30 participants for this study.

Do I have to take part?

No. It is up to you to decide whether or not you want to take part in this study. Please take your time to consider the inclusion criteria, and to make your decision; we will wait for at least 14 days before asking for your decision. If you do decide to take part you will be asked to sign a consent form. You can decide not to take part or to withdraw from the study until 14 days following data collection. If you wish to have your data withdrawn please contact the researcher with your participant number (found on your copy of the consent form) and your data will then not be used.

What will happen if I agree to take part?

If you agree to take part:

1. You and the Lead Researcher will arrange a mutually convenient time to come into the University laboratory (EEG052) on St John's Campus to undergo the various measurements and tests required for this study. This appointment will need to be in the morning, preferably between the hours of 9am and 11am, and should take less than one hour. You will be asked to have had breakfast before this appointment.
2. While in the laboratory, you will be asked to complete a brief questionnaire, which will provide some basic research data and verify your eligibility. Two consent forms will be signed – one to be retained by you and the other to be returned to the Lead Researcher. You will be assigned a participant number (found on your consent form) so that all personal data can be kept anonymous**.**
3. You will be weighed and your height will be measured. Your body fat percentage and BMI will be ascertained using a bioelectrical impedance monitor. Your waist and hip measurements will be taken.
4. You will be asked to give two small samples of capillary blood from a finger. This will be done using a sterile lancet, and should cause minimal discomfort. One sample will be taken onto a $1cm^2$ piece of filter paper and will be tested to determine the levels of various fatty acids in your blood, by using gas chromatography. The second sample will be taken using a small capillary tube and will be used for a haematocrit test – to ascertain the percentage of blood cells by volume, and therefore the oxygen-carrying capacity of your blood. This provides important data on blood viscosity, a factor affecting cardiac output.
5. You will be asked to lie down on the laboratory bench, or on a mat on the floor. 4 ECG leads will be attached to electrodes placed onto each of your hands and feet. A reading will then be taken, to look at your heart's rhythm and electrical activity. You will not feel anything, and this should take only a few minutes. At the same time, a pulse oximeter will be placed on one of your fingers to measure your blood oxygen levels.
6. Your blood pressure will be taken using a blood pressure monitor.
7. You will be given a 4-day food diary and some guidance and information on how to accurately fill it in. You can chose a 4-day period to suit you, but the 4 days must be consecutive and should include two weekend days. As much information as possible should be provided on each food item/ingredient i.e. brand, variety etc, to give the most accurate analysis of fatty acid intake possible. Where possible, food items should be weighed (and specify whether weight is raw or cooked) and where this is not possible, an estimation of weight or portion size can be made (see information on portioning). Food diaries can be posted using the stamped addressed envelope provided. You cannot be included in the study if the

food diary is not completed and returned. Please contact the Lead Researcher if you are having any problems filling out the food diary.

The results of all of the above tests and measurements, and any other information you give, are confidential and anonymous.

If during any part of the laboratory testing you would like a break, or feel uncomfortable, please let the Lead Researcher know so that measures can be taken to make you more comfortable.

Please be aware that the Lead Researcher is not permitted to give feedback on results from tests, and cannot offer any dietary or medical advice as the Lead Researcher is not medically trained. The 4-lead ECG is not a diagnostic tool and has no clinical validity. It is for research purposes only. If at any point you feel concerned about any of the test results, please speak to a trained healthcare provider.

What are the benefits for me in taking part?

The results of the study will be of no direct benefit to you, however, you and other members of the public may benefit in future from the information learned from this study.

Are there any risks for me if I take part?

The research carries only a minor risk of infection from the blood sampling, but this risk is no greater that that expected in normal life. However, steps will be taken to minimise this risk. No major risks are associated with any of the other data collection methods.

What will you do with my blood samples?

All blood samples are stored/processed in accordance with the Human Tissue Act 2004, which states that without an licence (which the University of Worcester does not possess) human cells can only be stored for up to 48 hours, unless they are put through a process to render them acellular.

What will you do with my information?

Your personal data / information will be treated confidentially at all times; that is, it will not be seen by anyone other than the Lead Researcher, Study Supervisor or any third parties specified in the consent form unless it has been fully anonymised.

During the project, all data / information will be kept securely in line with the University's Policy for the Effective Management of Research Data and its Information Security Policy.

We will process your personal information for a range of purposes associated with the project primary of which are:

- To use your information along with information gathered from other participants in the research project to seek new knowledge and understanding that can be derived from the information we have gathered.
- To summarise this information in written form for the purposes of dissemination (through research reports, a thesis / dissertation, conference papers, journal

articles or other publications). Any information disseminated / published will be at a summary level and will be fully anonymised and there will be no way of identifying your individual personal information within the published results.

- To use the summary and conclusions arising from the research project for teaching and further research purposes. Any information used in this way will be at a summary level and will be fully anonymised. There will be no way of identifying your individual personal information from the summary information used in this way.

If you wish to receive a summary of the research findings or to be given access to any of the publications arising from the research, please contact the researcher.

How long will you keep my data for?

Your personal data will be retained until the project (including the dissemination period) has been completed (September 2020). Within three months of completion of the project, we destroy all data relating to the project.

How can I find out what information you hold about me?

You have certain rights in respect of the personal information the University holds about you. For more information about Individual Rights under GDPR and how you exercise them please visit: https://www.worcester.ac.uk/informationassurance/requests-for-personal-data.html.

What happens next?

Please keep this information sheet. If you do decide to take part, please contact the Lead Researcher using the details below.

Thank you for taking the time to read this information.

If you decide you want to take part in our project, and we hope you do, or if you have any further questions then please contact:

Lead Researcher: Georgie Sherrard
Sheg21_10@uni.worc.ac.uk
07903 824506

If you have any concerns about the project at this point or at any later date you may contact the **Lead Researcher** (contact as above) or you may contact the Study Supervisor:

Dr Allain Bueno
a.bueno@worc.ac.uk

If you would like to speak to an independent person who is not involved in this study, please contact Michelle Jellis at the University of Worcester, using the following details:

Michelle Jellis
Secretary to Research Ethics Panel for College of Health, Life and Environmental Sciences
University of Worcester, Henwick Grove,Worcester WR2 6AJ
ethics@worc.ac.uk

Participant ID no.:

University of Worcester

INFORMED CONSENT FORM (NON-NHS RESEARCH)

Title of Project **Are dietary fatty acids, blood fatty acid composition and anthropometric biomarkers associated with ventricular depolarisation? An observational study in a sample population of healthy pre-menopausal women.**

Name of Researcher Georgie Sherrard

I, the undersigned, confirm that (**please initial boxes as appropriate**):

1.	I have read and understood the information about the project, as provided in the Information Sheet dated 21st March 2019, or it has been read to me.	
2.	I have been able to ask questions about the project and my participation and my questions have been answered to my satisfaction.	
3.	I understand that taking part in this study involves completing an honest and accurate 4-day food diary as well as a number of laboratory-based tests: a 4-lead electrocardiogram (ECG), measurements to ascertain BMI and body fat percentage, blood pressure and blood oxygen level monitoring, and two samples of capillary blood, taken using a sterile lancet.	
4.	I understand that taking part in the study carries a minor risk of infection from the blood sampling, but that precautions will be taken to minimise this risk.	
5.	I understand I can withdraw any time up until 14 days after data collection without giving reasons and that I will not be penalised for withdrawing nor will I be questioned on why I have withdrawn.	
6.	I understand that the information I provide will be used for the purposes of dissemination through research reports, a thesis / dissertation, conference papers, journal articles or other publications.	
7.	The procedures regarding confidentiality have been clearly explained (e.g. use of names, pseudonyms, anonymisation of data, etc.) to me.	
8.	I understand that personal information collected about me that can identify me, such as my name, or where I live, will not be shared beyond the Lead Researcher and the Study Supervisor.	
9.	I understand that other researchers will have access to this data only if they agree to preserve the confidentiality of the data and if they agree to the terms I have specified in this form.	
10.	I understand that the Lead Researcher is not permitted to give feedback on results from tests, and cannot offer any dietary or medical advice.	
11.	I understand that my blood samples will be processed in accordance with the Human Tissue Act 2004.	
12.	I voluntarily agree to participate in the project.	
13.	I know who to contact if I have any concerns about this research	

Name of Participant	Signature	Date
Name of Researcher	Signature	Date

PARTICIPANT QUESTIONNAIRE

Are you:

Male? ☐ Female? ☐

What is your date of birth?

_____

Are you a smoker or have you been a regular smoker at some point in the past two years?

Yes ☐ No ☐

Have you been through the menopause?

Yes ☐ No ☐

Please tick if any of the following apply to you.

I am/have:

Type 2 diabetes ☐
Heart disease ☐
Liver, kidney or lung disease ☐
A pacemaker ☐
A psychiatric disorder ☐
Taking blood thinners? ☐
Taking beta-blockers or other medication that affects heart rhythm ☐
Previously fainted at the sight of my own blood ☐

Do you regularly take, or have you recently taken any medications?

Yes ☐ No ☐

If you are happy to state what these medications are, please comment below.

_____

Do you have a known allergy to methacrylates?
(Found in acrylic nails, paint, varnish, printing ink, adhesives, glue, orthopaedic prostheses, bone cement and dental restorative materials.)

Yes ☐ No ☐

Do you have a known allergy to any of the following?:

Rubber ☐ Latex ☐ Plasters ☐

MEASUREMENTS AND TEST RESULTS

Height (cm)_____

Weight (kg)_____

Results from the bioelectrical impedance test:

Body fat percentage_____ BMI_____

Waist and hip measurements:

Waist measurement_____

Hip measurement_____

Haematocrit result:

_____

Heart rate:

_____

Pulse oximeter reading: (after 1 minute)

_____

Blood pressure:

_____

(Attach ECG print-out to this document, including participant ID number.)

Participant number:

FOOD DIARY

Please fill this in as honestly and accurately as possible for 4 consecutive days (including two weekend days), giving as much detail as possible.

DAY	_____DAY		_____DAY	
	Food	Amount/weight	Food	Amount/weight
Breakfast				
Lunch				
Dinner				
Snacks and drinks				
Supplements				

DAY	_____DAY		_____DAY	
	Food	Amount	Food	Amount
Breakfast				
Lunch				
Dinner				
Snacks and drinks				
Supplements				

NB: Please feel free, if necessary, to use extra sheets of paper to complete your food diary. If you do so, please make sure your participant number (top right) is included on these sheets.

APPENDIX B

Identification of FAMEs

Commercial FAME standards were acquired from Merck-Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) (Tables B1, B2 and B3), and analysis was carried out as retention times of FAMEs within samples were compared to these known standards. Others in the group had previously carried out the methylation process (as per the process seen in the Methods section) where these standards were acquired in non-methyl form.

Table B1. No.1 Fatty acids standard mix (F.A.M.E. Mix, C4-C24) (Sigma-Aldrich (Merck KGaA, Darmstadt, Germany) used for the detection of fatty acid methyl esters by Gas Chromatography - Fame Ionisation Detection (GC-FID). Table reproduced with permission from E.C Joyce (2022).

F.A.M.E. Mix, C4-C24 (Sigma-Aldrich (Merck KGaA, Darmstadt, Germany)		Code
C4:0	Methyl butyrate 4 wt. %	18919
C6:0	Methyl hexanoate 4 wt. %	
C8:0	Methyl octanoate 4 wt. %	
C10:0	Methyl decanoate 4 wt. %	
C11:0	Methyl undecanoate 2 wt. %	
C12:0	Methyl dodecanoate 4 wt. %	
C13:0	Methyl tridecanoate 2 wt. %	
C14:0	Methyl myristate 4 wt. %	
C14:1	Methyl myristoleate 2 wt. %	
C15:0	Methyl pentadecanoate 2 wt. %	
C15:1	Methyl *cis*-10-pentadecenoate 2 wt. %	
C16:0	Methyl palmitate 6 wt. %	
C16:1n7	Methyl palmitoleate 2 wt. %	
C17:0	Methyl heptadecanoate 2 wt. %	
C17:1	Methyl *cis*-10-heptadecenoate 2 wt. %	
C18:0	Methyl stearate 4 wt. %	
C18:1n9 *trans*	Methyl elaidate 2 wt. %	
C18:1n9	Methyl oleate 4 wt. %	
C18:2n6t	Methyl linolelaidate 2 wt. %	
C18:2n6	Methyl linoleate 2 wt. %	
C18:3n6	Methyl γ-linolenate 2 wt. %	
C18:3n3	Methyl linolenate 2 wt. %	
C20:0	Methyl arachidate 4 wt. %	
C20:1n9	Methyl *cis*-11-eicosenoate 2 wt. %	
C21:0	Methyl heneicosanoate 2 wt. %	
C20:2n6	*cis*-11,14-Eicosadienoic acid methyl ester 2 wt. %	
C20:3n6	*cis*-8,11,14-Eicosatrienoic acid methyl ester 2 wt. %	
C20:4n6	Methyl arachidonate 2 wt. %	
C20:3n3	*cis*-11,14,17-Eicosatrienoic acid methyl ester 2 wt. %	
C22:0	Methyl behenate 4 wt. %	
C22:1n9	Methyl *cis*-13-docosenoate 2 wt. %	
C20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester 2 wt. %	
C23:0	Methyl tricosanoate 2 wt. %	
C22:2n6	*cis*-13,16-Docosadienoic acid methyl ester 2 wt. %	
C24:0	Methyl tetracosanoate 4 wt. %	
C24:1n9	Methyl *cis*-15-tetracosenoate 2 wt. %	
C22:6n3	*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester 2 wt. %	

Table B2. FA standard mixes (PUFA No.3; Linolenic Acid Methyl Ester Isomer mix; Linoleic Acid Methyl Ester Isomer Mix; F.A.M.E. Mix, C20:1-C20:5 Unsaturates) used for the detection of fatty acid methyl esters by Gas Chromatography - Fame Ionisation Detection (GC-FID). Table reproduced with permission from E.C Joyce (2022).

PUFA No.3 - From Menhaden Oil, analytical standard *		47085-U
C22:5n3	*cis*-4,7,10,13,16,19-Docosahexaenoic acid methyl ester	
C20:3n3	*cis*-11,14,17-Eicosatrienoic acid methyl ester	
C20:4n6	Methyl arachidonate	
C22:5n3	Methyl all-*cis*-7,10,13,16,19-docosapentaenoate	
C20:5n3	Methyl all-*cis*-5,8,11,14,17-eicosapentaenoate	
20:1n11	Methyl *cis*-11-eicosenoate	
C18:2n6	Methyl linoleate	
C18:3n3	Methyl linolenate	
C14:0	Methyl myristate	
C18:1n9	Methyl oleate	
C16:0	Methyl palmitate	
C16:1n7	Methyl palmitoleate	
C18:0	Methyl stearate	
18:4n3	Methyl stearidonate	
18:2n4	11,14-Octadecadienoic acid methyl ester	
18:3n4	9,11,14-Octadecatrienoic acid methyl ester	
C18:1n7	*cis*-11-Octadecenoic methyl ester	
Linolenic Acid Methyl Ester Isomer mix		CRM47792
C18:3n3 (*cis* and *trans*)	*cis*-9, *cis*-12,*cis*-15-Octadecatrienoic acid methyl ester 3% (w/w)	
	cis-9, *cis*-12,*trans*-15-Octadecatrienoic acid methyl ester 7% (w/w)	
	cis-9, *trans*-12,*cis*-15-Octadecatrienoic acid methyl ester 7% (w/w)	
	cis-9, *trans*-12,*trans*-15-Octadecatrienoic acid methyl ester 15% (w/w)	
	trans-9, *cis*-12,*cis*-15-Octadecatrienoic acid methyl ester 7% (w/w)	
	trans-9, *cis*-12,*trans*-15-Octadecatrienoic acid methyl ester 15% (w/w)	
	trans-9, *trans*-12,*trans*-15-Octadecatrienoic acid methyl ester 30% (w/w)	
Linoleic Acid Methyl Ester Isomer Mix		CRM47791
C18:2n6 (*Cis* and *trans*)	*cis*-9, *cis*-12-Octadecadienoic acid methyl ester 10 % (w/w)	
	cis-9, *trans*-12-Octadecadienoic acid methyl ester 20 % (w/w)	
	trans-9, *cis*-12-Octadecadienoic acid methyl ester 20 % (w/w)	
	trans-9,12-Octadecadienoic acid methyl ester 50 % (w/w)	
	cis-9, *cis*-12-Octadecadienoic acid methyl ester 10 % (w/w)	
	cis-9, *trans*-12-Octadecadienoic acid methyl ester 20 % (w/w)	
	trans-9, *cis*-12-Octadecadienoic acid methyl ester 20 % (w/w)	
F.A.M.E. Mix, C20:1-C20:5 Unsaturates		18913
C20:1n9	*cis*-11-Eicosenoic acid methyl ester ~ 10 mg	
C20:2n6	*cis*-11,14-Eicosadienoic acid methyl ester ~ 10 mg	
C20:3n3	*cis*-11,14,17-Eicosatrienoic acid methyl ester ~ 10 mg	
C20:4n6	*cis*-5,8,11,14-Eicosatetraenoic acid methyl ester ~ 10 mg	
C20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester ~ 10 mg	

Table B3. Individual FA standard used for the detection of fatty acid methyl esters by Gas Chromatography - Fame Ionisation Detection (GC-FID). Table reproduced with permission from E.C Joyce (2022).

C16:1n7	palmitoleic acid / *cis*-9-Hexadecenoic acid *	P9417
C17:0	Heptadecanoic acid *	H3500
C20:1n9	*cis*-11-Eicosenoic acid *	44878
C20:2n6	*cis*-11,14-Eicosadienoic acid methyl ester	E7477
C20:3n6	*cis*-8,11,14-Eicosatrienoic acid methyl ester	E3511
C20:4n6	Methyl arachidonate	A9298
C22:0	Docosanoic acid / Behenic acid *	216941
C22:1n9	*cis*-13-Docosenoic acid / Erucic acid *	45629
C20:5n3	*cis*-5,8,11,14,17-Eicosapentaenoic acid methyl ester	47571-U
C22:2n6	*cis*-13,16-Docosadienoic acid methyl ester	D4034
C24:1n9	*cis*-15-Tetracosenoic acid / Nervonic acid *	N1514
C22:6n3	*cis*-4,7,10,13,16,19-Docosahexaenoic acid *	D2534
DMA 16:0	16:0 dimethylacetal *	852446C
DMA 18:0	18:0 dimethylacetal *	852448C
DMA 18:1	18:1 dimethylacetal *	852449C
C18:1n7t	*trans*-Vaccenic acid / 11-*trans*-Octadecenoic acid *	V1131
C18:1n7	*cis*-vaccenic acid / *cis*-11-Octadecenoic acid *	V0384
C22:4n6	*cis*-7,10,13,16-Docosatetraenoic acid *	D3659
C22:5n3	*cis*-7,10,13,16,19-Docosapentaenoic methyl ester	47563-U
C20:3n9	*cis*-5,8,11-Eicosatrienoic acid, Mead acid	43059

*Indicates where FA standards were acquired in non-methyl form and subjected to methylation by others in the group.

APPENDIX C

Raw data from all tests performed on participants.

Table C1: Data collected from the anthropometric and laboratory tests performed on each participant.

Participant number	Age	Height (cm)	Weight (kg)	Waist circ. (cm)	Hip circ. (cm)	Waist:hip ratio	Body fat %	BMI	Haematocrit (%)	Heart rate (bpm)	Pulse ox (%)	Blood pressure (mmHg)	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)
2	40	170.2	73.0	81.5	105.0	0.78	35.4	25.30	38	60	98	103/77	103	77	85.67
3	35	153.0	90.5	94.0	116.0	0.81	44.6	38.70	37	68	97	114/71	114	71	85.33
4	40	173.5	83.5	84.0	110.0	0.76	38.8	27.70	38	65	98	109/68	109	68	81.67
5	35	169.4	60.0	71.0	101.0	0.70	30.8	20.90	40	78	98	126/69	126	69	88.00
6	30	174.3	74.0	79.0	108.0	0.73	34.3	24.30	44	63	97	109/71	109	71	83.67
7	40	158.5	55.5	72.0	95.5	0.75	30.0	22.10	35	72	98	124/71	124	71	88.67
8	38	159.7	45.0	65.0	88.0	0.74	32.7	17.70	41	65	98	104/70	104	70	81.33
9	40	162.7	55.5	75.0	95.0	0.79	29.4	21.00	42	68	98	114/78	114	78	90.00
10	30	165.5	71.5	89.0	103.0	0.86	38.1	26.10	40	63	98	109/69	109	69	82.33
11	39	160.0	63.5	82.5	97.0	0.85	37.0	24.80	39	61	98	95/71	95	71	79.00
12	30	158.5	86.5	93.0	118.0	0.79	45.7	34.40	40	68	97	125/87	125	87	99.67
13	36	168.0	58.5	70.0	93.5	0.75	34.1	20.70	39	54	98	106/69	106	69	81.33
14	31	168.0	97.5	91.0	128.0	0.71	46.7	34.50	40	59	99	116/72	116	72	86.33
15	25	172.5	93.0	87.0	111.0	0.78	40.0	31.30	41	83	98	126/88	126	88	100.67
16	38	174.0	80.0	82.0	108.0	0.76	35.8	24.40	38	64	99	128/73	128	73	91.33
17	36	164.5	66.5	70.0	102.0	0.69	34.1	24.50	40	55	98	119/89	119	89	99.00
18	39	171.0	66.0	73.0	98.0	0.74	28.5	22.60	35	68	99	125/85	125	85	98.33
19	36	178.0	85.0	89.0	112.0	0.79	38.9	26.80	43	81	99	125/73	125	73	90.33
20	38	171.5	65.0	75.0	99.0	0.76	33.4	22.20	40	71	98	107/67	107	67	80.33
21	40	165.5	69.0	82.0	102.5	0.80	33.2	25.20	38	82	97	128/82	128	82	97.33
22	37	174.0	69.5	80.0	106.0	0.75	36.9	23.00	41	78	98	125/86	125	86	99.00
23	39	170.0	95.0	88.0	112.0	0.79	43.0	32.90	37	67	98	131/92	131	92	105.00
24	38	152.5	51.5	68.0	90.0	0.76	33.2	22.10	43	69	98	135/92	135	92	106.33

Abbreviations: AA = arachidonic acid; ALA = alpha-linolenic acid; BMI = body mass index; bpm = beats per minute; Circ. = circumference; DBP = diastolic blood pressure; DHA = docosahexaenoic acid; EPA = eicosapentaenoic acid; LA = linoleic acid; MAP = mean arterial pressure; MAP = DBP + 1/3 (SBP-DBP); PUFA = polyunsaturated fatty acid; Pulse ox = oxygen saturation level of the blood; SBP = systolic blood pressure.

Table C2: ECG measurements for all participants for each of the sections of the cardiac conduction system under investigation. Values are averages taken from at least 20 individual heart beats.

Participant number	AUC of QRS complex (mm^2)	PR interval*		QRS duration*		P wave duration*		QT duration*		ARP*		R wave amplitude**		P wave amplitude**	
		mm	ms	Mm	ms	mm	ms	mm	ms	mm	ms	Mm	mV	mm	mV
2	6.79	4.08	163.2	1.81	72.4	2.60	104.0	9.90	396.0	8.00	320.0	10.68	1.068	0.92	0.092
3	7.78	3.66	146.4	2.24	89.6	2.75	110.0	10.07	402.8	7.69	307.6	11.74	1.174	1.72	0.172
4	10.47	4.65	186.0	2.26	90.4	2.43	97.2	9.49	379.6	7.59	303.6	12.65	1.265	1.27	0.127
5	5.78	4.21	168.4	1.66	66.4	2.65	106.0	9.51	380.4	7.70	308.0	9.09	0.909	2.30	0.230
6	8.76	3.12	124.8	2.33	93.2	2.25	90.0	9.76	390.4	7.46	298.4	10.28	1.028	1.35	0.135
7	12.65	3.50	140.0	2.16	86.4	2.18	87.2	9.39	375.6	7.66	306.4	17.88	1.788	1.33	0.133
8	5.60	3.24	129.6	1.87	74.8	1.99	79.6	9.80	392.0	7.79	311.6	8.07	0.807	1.20	0.120
9	10.88	3.26	130.4	1.87	74.8	2.18	87.2	8.73	349.2	6.86	274.4	15.74	1.574	1.27	0.127
10	7.06	3.58	143.2	2.19	87.6	2.51	100.4	10.03	401.2	7.99	319.6	8.58	0.858	1.02	0.102
11	5.77	3.37	134.8	1.98	79.2	2.28	91.2	9.81	392.4	7.87	314.8	7.70	0.770	0.94	0.094
12	9.33	4.28	171.2	1.85	74.0	2.08	83.2	10.15	406.0	8.20	328.0	13.91	1.391	1.48	0.148
13	7.66	3.19	127.6	1.73	69.2	2.02	80.8	10.08	403.2	8.11	324.4	11.54	1.154	1.58	0.158
14	11.73	3.81	152.4	2.34	93.6	2.38	95.2	9.76	390.4	7.51	300.4	16.58	1.658	1.45	0.145
15	5.04	4.25	170.0	2.32	92.8	2.56	102.4	9.06	362.4	6.97	278.8	4.84	0.484	1.62	0.162
16	10.40	4.49	179.6	2.24	89.6	2.67	106.8	9.73	389.2	7.40	296.0	12.69	1.269	1.65	0.165
17	7.52	3.71	148.4	2.12	84.8	2.31	92.4	9.90	396.0	8.15	326.0	9.70	0.970	1.38	0.138
18	7.35	4.28	171.2	1.83	73.2	2.58	103.2	9.36	374.4	7.61	304.4	11.54	1.154	1.42	0.142
19	6.67	4.45	178.0	2.31	92.4	2.83	113.2	10.47	418.8	7.90	316.0	8.70	0.870	1.77	0.177
20	6.06	3.68	147.2	2.13	85.2	2.19	87.6	10.71	428.4	7.99	319.6	8.41	0.841	1.07	0.107
21	5.79	3.04	121.6	1.74	69.6	1.94	77.6	9.88	395.2	7.82	312.8	9.01	0.901	1.07	0.107
22	6.21	2.77	110.8	2.12	84.8	1.44	57.6	10.84	433.6	8.73	349.2	8.68	0.868	0.67	0.067
23	4.58	3.82	152.8	1.76	70.4	2.60	104.0	10.44	417.6	8.23	329.2	7.70	0.770	1.19	0.119
24	8.16	3.31	132.4	1.86	74.4	2.23	89.2	9.14	365.6	7.14	285.6	12.31	1.231	1.36	0.136

Participant 1 was eliminated due to an abnormal ECG. *1mm = 0.04 seconds, so times in milliseconds (ms) were calculated. **1mm = 0.1mV (millivolts), so voltages were calculated. Abbreviations: ARP = Absolute refractory period; AUC = Area under the curve

Table C3: Average daily intakes, ascertained by dietary analysis, for each participant over a four-day period.

Participant Number	Energy (kcal)	Total carbohydrates (g)	Total protein (g)	Total fat (g)	SFA (g)	MUFA (g)	PUFA (g)	n-6 total (g)	n-3 total (g)	n-6:n-3 ratio	TFA (g)	Cholesterol (mg)
2	1876.43	199.95	79.07	63.45	17.59	15.69	6.58	2.47	0.69	3.58	0.30	386.29
3	1415.45	124.68	68.66	69.04	26.01	6.75	4.50	1.27	0.34	3.74	0.26	103.61
4	1850.73	215.16	69.87	78.96	24.39	18.23	14.79	10.29	2.19	4.70	0.24	108.17
5	1596.15	188.67	76.84	42.07	16.20	9.86	3.86	1.96	0.46	4.26	0.39	156.85
6	1746.24	146.82	85.67	90.71	30.20	30.20	10.41	4.65	0.94	4.95	0.76	356.98
7	1889.13	196.69	71.86	90.38	26.95	21.05	9.81	2.79	1.57	1.78	0.90	203.18
8	794.57	96.10	35.85	29.66	9.45	10.08	4.22	1.45	0.25	5.80	0.24	52.17
9	2224.76	278.34	81.86	87.02	42.71	13.10	3.85	2.59	0.69	3.75	0.59	84.69
10	1250.80	113.34	68.98	57.90	17.91	13.29	9.21	8.20	0.99	8.28	0.24	87.92
11	1721.61	180.93	75.45	64.88	23.74	15.96	8.87	4.07	1.53	2.66	1.03	182.43
12	1604.80	143.38	66.69	52.17	19.06	10.63	3.79	2.35	0.25	9.40	0.49	74.31
13	1827.07	228.17	68.08	70.92	26.36	17.93	7.07	2.00	0.34	5.88	0.93	281.52
14	1945.24	231.17	76.41	78.76	27.95	22.29	9.45	2.07	0.34	6.09	0.19	395.43
15	1552.18	169.66	74.93	56.70	22.43	16.07	8.50	3.87	0.50	7.74	0.55	246.93
16	1952.60	209.09	84.69	75.22	27.77	16.01	5.95	3.48	0.49	7.10	0.35	314.92
17	1044.36	25.25	33.48	89.88	36.30	25.87	9.73	5.70	2.44	2.34	0.44	83.30
18	2077.68	236.46	85.89	87.34	24.62	30.21	14.69	3.01	1.50	2.01	0.82	219.65
19	706.13	51.41	42.73	27.89	10.31	8.89	2.31	0.86	0.24	3.58	0.21	53.22
20	1730.76	205.29	66.57	71.53	32.68	4.44	3.66	3.07	0.43	7.14	0.27	134.26
21	1157.62	61.02	75.83	53.21	16.56	16.98	9.85	4.55	3.30	1.38	0.33	498.67
22	1179.58	120.45	60.64	50.54	18.28	14.49	6.38	3.53	0.15	23.53	0.21	242.56
23	1250.42	143.45	67.04	32.14	11.24	3.59	2.81	0.67	0.81	0.83	0.02	90.46
24	1894.70	214.66	101.41	64.00	29.75	17.48	4.26	2.82	0.40	7.05	0.43	222.86

Abbreviations: Kcal = kilocalories; MUFA = monounsaturated fatty acid; n-3 = PUFA from the omega 3 family; n-6 = PUFA from the omega 6 family; PUFA = polyunsaturated fatty acid; SFA = saturated fatty acid; TFA = trans fatty acid.

Table C4: Percentages of each FA found in blood samples of all participants, ascertained by GC-FID.

Participant number	C14:0	C16:0	C18:0	C20:0	C22:0	C24:0	Total % SFA	C16:1n-7	C18:1n-9	C18:1n7	C20:1n-9	C22:1n-9	C24:1n-9	Total % MUFA	C18:2n-6	C18:3n-6
2	0.485	22.70	9.31	0.083	0.349	1.66	34.58	1.108	18.57	1.312	0.235	0.062	1.573	22.86	25.80	0.138
3	0.357	23.17	9.96	0.147	0.535	1.97	36.14	1.067	23.40	1.120	0.206	0.033	1.269	27.10	21.25	0.135
4	0.753	24.78	9.87	0.111	0.434	2.23	38.17	1.224	21.06	0.971	0.790	0.081	1.458	25.58	19.18	0.339
5	0.266	24.23	11.33	0.149	0.405	2.33	38.71	1.127	18.97	1.713	0.382	0.133	1.843	24.17	20.29	0.106
6	0.646	19.58	10.38	0.202	0.289	2.12	33.21	0.574	19.47	1.348	0.389	0.151	1.907	23.83	25.98	0.162
7	0.843	22.56	9.92	0.104	0.385	1.92	35.73	0.550	20.12	1.103	0.437	0.076	1.463	23.75	27.12	0.124
8	0.253	23.15	11.50	0.184	0.669	2.26	38.01	0.523	19.95	1.331	0.319	0.053	1.810	23.98	18.94	0.068
9	1.131	25.84	9.90	0.161	0.387	2.01	39.43	1.536	22.12	1.624	0.318	0.070	1.447	27.11	18.58	0.053
10	0.545	23.07	9.36	0.174	0.334	1.88	35.36	0.786	22.69	1.483	0.434	0.053	1.546	26.99	21.70	0.089
11	0.732	22.92	9.10	0.137	0.128	1.86	34.87	0.798	22.17	1.721	0.408	0.058	1.827	26.98	22.65	0.166
12	1.259	29.50	7.68	0.058	0.209	1.43	40.14	3.256	21.16	1.672	0.219	0.057	0.803	27.17	18.72	0.262
13	0.535	22.88	10.07	0.095	0.273	2.42	36.27	0.535	18.57	1.664	0.411	0.033	1.599	22.81	21.49	0.067
14	0.327	21.38	10.82	0.155	0.434	2.33	35.43	0.676	16.89	1.231	0.256	0.054	1.538	20.64	26.17	0.117
15	0.738	24.29	8.73	0.109	0.390	1.78	36.03	1.902	24.54	2.005	0.300	0.058	1.185	29.99	21.28	0.049
16	0.760	22.56	8.83	0.111	0.286	2.23	34.78	0.728	19.47	1.586	0.350	0.040	1.494	23.67	22.95	0.137
17	1.041	22.43	7.57	0.079	0.307	1.40	32.82	0.481	16.86	0.934	0.336	0.021	0.862	19.50	32.32	0.128
18	0.585	23.23	8.71	0.090	0.123	1.80	34.53	0.910	17.61	1.549	0.262	0.048	1.364	21.74	24.30	0.152
19	0.780	25.41	10.46	0.106	0.206	1.99	38.96	1.599	21.57	2.301	0.473	0.058	1.928	27.93	17.88	0.083
20	0.745	19.04	8.16	0.087	0.210	3.17	31.41	0.626	20.36	1.538	0.494	0.098	2.270	25.39	21.04	0.133
21	0.976	23.80	5.88	0.050	0.126	1.28	32.11	1.176	19.13	1.619	0.486	0.098	1.255	23.76	26.56	0.102
22	2.084	23.07	2.21	0.134	0.156	1.24	28.89	2.027	26.31	2.287	0.644	0.072	0.997	32.33	21.52	0.134
23	1.195	25.35	6.80	0.052	0.117	1.41	34.93	3.260	21.50	1.819	0.369	0.072	1.304	28.33	20.21	0.146
24	2.044	21.69	5.53	0.068	0.244	1.99	31.56	1.228	18.61	1.866	0.614	0.048	1.459	23.83	25.98	0.140

Abbreviations: Kcal = kilocalories; MUFA = monounsaturated fatty acid; SFA = saturated fatty acid.

Table C4 continued.

Participant number	C20:2n-6	C20:3n-6	C20:4n-6	Total % n-6	C18:3n-3	C20:3n-3	C20:5n-3	C22:5n-3	C22:6n-3	Total % n-3	Total % PUFA	n-6:n:3 ratio	DMA 16:0	DMA 18:0 A	DMA 18:0 B
2	0.096	1.112	8.25	35.40	0.342	0.149	0.564	0.953	3.64	5.65	41.05	6.26	0.349	0.394	0.765
3	0.081	1.000	8.32	30.78	0.192	0.154	0.430	0.759	2.82	4.35	35.13	7.07	0.262	0.392	0.980
4	0.095	1.057	9.23	29.90	0.295	0.195	0.657	1.046	2.68	4.877	34.77	6.13	0.246	0.357	0.867
5	0.128	1.269	8.57	30.37	0.209	0.221	0.432	0.976	3.15	4.99	35.36	6.09	0.305	0.434	1.027
6	0.092	1.111	8.67	36.01	0.352	0.555	1.231	0.735	2.27	5.14	41.15	7.00	0.244	0.267	1.292
7	0.123	1.329	6.33	35.03	0.709	0.165	0.336	0.855	2.03	4.10	39.13	8.55	0.254	0.267	0.875
8	0.110	0.877	10.07	30.07	0.258	0.161	0.423	1.247	3.75	5.83	35.90	5.15	0.374	0.585	1.144
9	0.122	1.875	7.07	27.70	0.228	0.184	0.262	0.970	2.25	3.90	31.60	7.11	0.267	0.452	1.144
10	0.107	1.445	7.72	31.06	0.293	0.243	0.381	0.892	2.99	4.80	35.86	6.47	0.323	0.403	1.070
11	0.108	1.294	8.14	32.36	0.215	0.264	0.405	0.801	2.40	4.09	36.45	7.92	0.335	0.283	1.084
12	0.107	1.456	7.53	28.08	0.315	0.113	0.609	0.972	1.44	3.45	31.53	8.14	0.256	0.250	0.657
13	0.102	1.414	10.08	33.15	0.221	0.339	0.409	1.200	3.46	5.63	38.78	5.89	0.418	0.525	1.199
14	0.083	1.126	10.12	37.62	0.180	0.176	0.408	1.093	2.49	4.35	41.97	8.65	0.357	0.457	1.142
15	0.130	1.243	6.00	28.70	0.390	0.124	0.280	0.731	2.34	3.86	32.57	7.43	0.283	0.379	0.750
16	0.109	1.165	10.40	34.76	0.333	0.238	0.639	1.082	2.98	5.27	40.03	6.59	0.246	0.330	0.945
17	0.057	0.429	7.55	40.48	0.338	0.129	0.829	0.929	3.77	6.00	46.48	6.75	0.199	0.316	0.698
18	0.096	1.552	10.95	37.05	0.161	0.208	0.641	1.050	3.00	5.05	42.11	7.33	0.358	0.386	0.874
19	0.099	1.042	8.02	27.13	0.065	0.524	0.343	0.693	2.68	4.31	31.43	6.30	0.320	0.347	1.014
20	0.088	1.404	12.35	35.01	0.145	0.630	0.343	1.352	3.42	5.88	40.90	5.95	0.392	0.581	1.330
21	0.090	1.384	8.45	36.59	0.391	0.289	0.572	0.833	4.12	6.20	42.80	5.90	0.211	0.303	0.818
22	0.082	1.550	9.57	32.86	0.201	0.469	0.401	0.742	3.12	4.93	37.79	6.66	0.238	0.221	0.532
23	0.075	1.222	8.54	30.19	0.520	0.211	1.043	0.939	2.69	5.40	35.58	5.59	0.242	0.290	0.632
24	0.086	1.343	9.75	37.30	0.291	0.252	0.512	1.409	3.41	5.87	43.17	6.36	0.316	0.325	0.800

Abbreviations: DMA = di-methyl acetal; PUFA = polyunsaturated fatty acid.

APPENDIX D

All statistically significant correlations between all variables.

Table D1: Variables showing a statistically significant association with the AUC of the QRS

Variable correlating with AUC of the QRS (mm^2)	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	r = 0.574	p = 0.04
Carbohydrates (g)*	r = 0.452	p = 0.03
Total fat (g)*	r = 0.661	p = <0.001
SFA (g)*	r = 0.549	p = 0.007
% of energy as protein*	r = -0.492	p = 0.017
% C18:1n-7**	r = -0.460	p = 0.022
% C22:6n-3**	r = -0.463	p = 0.026
n-6:n-3 ratio**	r = 0.576	p = 0.004

Table D2: Variables showing a statistically significant association with the QRS duration

Variable correlation with QRS duration (ms)	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.427	p = 0.047
BMI	rho = 0.418	p = 0.047
Waist circumference (cm)	r = 0.428	p =0.042
Hip circumference (cm)	r = 0.492	p = 0.017
% of energy as fat*	rho = 0.468	p = 0.024
% of energy as SFA*	rho = 0.446	p = 0.033

Table D3: Variables showing a statistically significant association with the R wave amplitude

Variable correlating with R wave amplitude (mV)	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	r = 0.593	p = 0.003
Carbohydrates (g)*	r = 0.497	p = 0.016
Total fat (g)*	r = 0.573	p = 0.04
SFA (g)*	r = 0.480	p = 0.021
% of energy as protein*	r = -0.468	p = 0.024
% C18:1n-7**	r = -0.440	p = 0.036
n-6:n-3 ratio**	rho = 0.423	p = 0.045

Table D4: Variables showing a statistically significant association with the PR interval

Variable correlating with PR interval (ms)	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.475	p = 0.026
Hip circ. (cm)	r = 0.437	p = 0.037
Total % SFA**	r = 0.504	p = 0.014

Table D5: Variables showing a statistically significant association with the P wave duration

Variable correlating with P wave duration (ms)	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.43	p = 0.046
Hip circ. (cm)	rho = 0.467	p = 0.025
% C20:3n-6**	rho = -0.439	p = 0.036

Table D6: Variables showing a statistically significant association with the P wave amplitude

Variable correlating with P wave amplitude (mV)	Pearson's/Spearman's correlation coefficient	Level of significance
Total % SFA**	r = 0.547	p = 0.007

Table D7: Variables showing a statistically significant association with the QT duration

Variable correlating with QT duration (ms)	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	r = -0.549	p = 0.007
Carbohydrates (g)*	r = -0.489	p = 0.018
Protein (g)*	rho = -0.709	p = <0.001
Total fat (g)*	r = -0.426	p = 0.043
SFA (g)*	r = -0.425	p = 0.043
MUFA (g)*	r = -0.422	p = 0.045
TFA (g)*	rho = -0.454	p = 0.029
% C20:2n-6**	r = -0.506	p = 0.014

Table D8: Variables showing a statistically significant association with the Absolute Refractory Period (ARP)

Variable correlating with ARP (mV)	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	rho = -0.575	p = 0.004
Carbohydrates (g)*	r = -0.485	p = 0.019
Protein (g)*	rho = -0.695	p = <0.001
SFA (g)*	r = -0.457	p = 0.028
% C20:2n-6**	r = -0.46	p = 0.027

Table D9: Variables showing a statistically significant association with energy intake

Variable correlating with energy (kcal)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Carbohydrates (g)*	r = 0.921	p = <0.001
Protein (g)*	r = 0.789	p = <0.001
Total fat (g)*	rho = 0.651	p = 0.001
SFA (g)*	rho = 0.604	p = 0.003
TFA (g)*	r = 0.473	p = 0.023
Cholesterol (mg)*	rho = 0.421	p = 0.047
% of energy as carbohydrate*	rho = 0.477	p = 0.022
% of energy as protein*	rho = -0.521	p = 0.012
n-6:n-3 ratio**	r = 0.436	p = 0.038
AUC of QRS (mm^2)	r = 0.574	p = 0.04
R wave amplitude (mV)	r = 0.593	p = 0.003
QT duration (ms)	r = -0.549	p = 0.007
ARP (ms)	rho = -0.575	p = 0.004

Table D10: Variables showing a statistically significant association with carbohydrate intake

Variable correlating with carbohydrate (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	r = 0.921	p = <0.001
Protein (g)*	r = 0.7	p = <0.001
Total fat (g)*	rho = 0.513	p = 0.013
SFA (g)*	rho = 0.503	p = 0.016
% of energy as carbohydrate*	rho = 0.716	p = <0.001
% of energy as protein*	rho = -0.49	p = 0.018
% of energy as MUFA*	r = -0.497	p = 0.016
% of energy as PUFA*	r = -0.424	p = 0.044
%C24:0**	r = 0.474	p = 0.022
%C20:3n-6**	r = 0.558	p = 0.006
%C22:5n-3**	r = 0.445	p = 0.033
AUC of QRS (mm^2)	r = 0.452	p = 0.03
R wave amplitude (mV)	r = 0.497	p = 0.016
QT duration (ms)	r = -0.489	p = 0.018
ARP (ms)	r = -0.485	p = 0.019

Table D11: Variables showing a statistically significant association with protein intake

Variable correlating with protein (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	r = 0.789	p = <0.001
Carbohydrates (g)*	r = 0.7	p = <0.001
Total fat (g)*	rho = 0.398	p = 0.061
MUFA (g)*	rho = 0.432	p = 0.041
Cholesterol (mg)*	r = 0.513	p = 0.012
QT duration (ms)	rho = -0.709	p = <0.001
ARP (ms)	rho = -0.695	p = <0.001

Table D12: Variables showing a statistically significant association with total fat intake

Variable correlating with total fat (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Heart rate (bpm)	r = -0.453	p = 0.03
Energy (kcal)*	rho = 0.651	p = 0.001
Carbohydrates (g)*	rho = 0.513	p = 0.013
Protein (g)*	rho = 0.398	p = 0.061
SFA (g)*	rho = 0.852	p = <0.001
MUFA (g)*	r = 0.695	p = <0.001
PUFA (g)*	r = 0.597	p = 0.003
n-6 (g)*	rho = 0.448	p = 0.033
n-3 (g)*	rho = 0.503	p = 0.014
TFA (g)*	r = 0.518	p = 0.011
% energy as protein*	r = -0.635	p = 0.001
% energy as fat*	rho = 0.57	p = 0.005
AUC of QRS (mm^2)	r = 0.661	p = <0.001
R wave amplitude (mV)	r = 0.573	p = 0.04
QT duration (ms)	r = -0.426	p = 0.043
% C16:0**	rho = -0.499	p = 0.016
% C16:1n-7**	r = -0.499	p = 0.015
% C18:1n-7**	r = -0.591	p = 0.003
Total % MUFA**	r = -0.485	p = 0.019
% C18:2n-6**	r = 0.551	P = 0.006
Total % n-6**	r = 0.551	p = 0.006
n-6:n-3**	r = 0.498	p = 0.016

Table D13: Variables showing a statistically significant association with SFA intake

Variable correlating with SFA (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	rho = 0.604	p = 0.003
Carbohydrates (g)*	rho = 0.503	p = 0.016
Total fat (g)*	rho = 0.852	p = <0.001
% of energy as protein*	r = -0.613	p = 0.002
% of energy as SFA*	rho = 0.651	p = 0.001
% C16:0**	rho = -0.549	p = 0.007
% n-6**	rho = 0.452	p = 0.032
AUC of QRS (mm^2)	r = 0.549	p = 0.007
R wave amplitude (mV)	r = 0.48	p = 0.021
QT duration (ms)	r = -0.425	p = 0.043
ARP (ms)	r = -0.457	p = 0.028

Table D14: Variables showing a statistically significant association with MUFA intake

Variable correlating with MUFA (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Protein (g)*	rho = 0.432	p = 0.041
Total fat (g)*	r = 0.695	p = <0.001
SFA (g)*	r = 0.408	p = 0.053
PUFA (g)*	r = 0.787	p = <0.001
n-6 (g)*	rho = 0.541	p = 0.009
n-3 (g)*	rho = 0.477	p = 0.021
TFA (g)*	r =-0.542	p = 0.008
Cholesterol (mg)*	r = 0.471	p = 0.023
% of energy as fat*	rho = 0.421	p = 0.047
% of energy as MUFA*	rho = 0.649	p = 0.001
% of energy as PUFA*	r = 0.611	p = 0.002
% of energy as TFA*	r = 0.488	p = 0.018
% C18:1n-9**	r = -0.478	p = 0.021
Total % MUFA**	r = -0.559	p = 0.006
% C18:2n-6**	r = 0.679	p = <0.001
Total % n-6**	r = 0.64	p = <0.001
QT duration (ms)	r = -0.422	p = 0.045

Table D15: Variables showing a statistically significant association with PUFA intake

Variable correlating with PUFA (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Total fat (g)*	r = 0.597	p = 0.003
MUFA (g)*	r = 0.787	p = <0.001
n-6 (g)*	rho = 0.682	p = <0.001
n-3 (g)*	rho = 0.663	p = <0.001
Cholesterol (mg)*	rho = 0.484	p = 0.02
% of energy as fat*	rho = 0.596	p = 0.003
% of energy as MUFA*	rho = 0.632	p = 0.002
% of energy as PUFA*	rho = 0.834	p = <0.001
% C18:1n-7**	r = -0.465	p = 0.025
% C18:2n-6**	rho = 0.666	p = <0.001
Total % n-6**	r = 0.439	p = 0.036

Table D16: Variables showing a statistically significant association with n-6 intake

Variable correlating with n-6 (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Total fat (g)*	rho = 0.448	p = 0.033
MUFA (g)*	rho = 0.541	p = 0.009
PUFA (g)*	rho = 0.682	p = <0.001
n-3 (g)*	rho = 0.624	p = 0.001
% of energy as PUFA*	rho = 0.65	p = 0.001
% C18:2n-6**	rho = 0.444	p = 0.035
Total % SFA**	rho = -0.467	p = 0.026

Table D17: Variables showing a statistically significant association with n-3 intake

Variable correlating with n-3 (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Haematocrit (%)	rho = -0.439	p = 0.036
Total fat (g)*	rho = 0.503	p = 0.014
MUFA (g)*	rho = 0.477	p = 0.021
PUFA (g)*	rho = 0.663	p = <0.001
n-6 (g)*	rho = 0.624	p = 0.001
n-6:n-3 ratio*	rho = -0.582	p = 0.004
% of energy as MUFA	r = 0.505	p = 0.014
% of energy as PUFA	rho = 0.507	p = 0.014
% C18:2n-6**	rho = 0.484	p = 0.019
% C18:3n-3**	rho = 0.539	p =0.008

Table D18: Variables showing a statistically significant association with intake ratio of n-6:n-3

Variable correlating with n-6:n-3 ratio*	Pearson's/Spearman's correlation coefficient	Level of significance
Haematocrit (%)	rho = 0.519	p = 0.011
n-3 (g)*	rho = -0.582	p = 0.004

Table D19: Variables showing a statistically significant association with TFA intake

Variable correlating with TFA (g)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = -0.473	p = 0.026
Hip circumference (cm)	r = -0.433	p = 0.039
Body fat %	r = -0.448	p = 0.032
BMI	rho = -0.424	p = 0.044
Energy (kcal)*	r = 0.473	p = 0.023
Total fat (g)*	r = 0.518	p = 0.011
SFA (g)*	r = 0.396	p = 0.061
MUFA (g)*	r = -0.542	p = 0.008
% of energy as TFA*	rho = 0.816	p = <0.001
n-6:n-3 ratio**	r = 0.452	p = 0.03
QT duration (ms)	rho = -0.454	p = 0.029

Table D20: Variables showing a statistically significant association with cholesterol intake

Variable correlating with cholesterol (mg)* intake	Pearson's/Spearman's correlation coefficient	Level of significance
Energy (kcal)*	rho = 0.421	p = 0.047
Protein (g)*	r = 0.513	p = 0.012
MUFA (g)*	r = 0.471	p = 0.023
PUFA (g)*	rho = 0.484	p = 0.02
Total % SFA**	r = -0.465	p = 0.025
Total % MUFA**	r = -0.429	p = 0.042
% C18:2n-6**	rho = 0.639	p = 0.001

Table D21: Variables showing a statistically significant association with % of energy as carbohydrate

Variable correlating with % of energy as carbohydrate*	Pearson's/Spearman's correlation coefficient	Level of significance
BMI	rho = -0.421	p = 0.046
Energy (kcal)*	rho = 0.477	p = 0.022
Carbohydrate (g)*	rho = 0.716	p = <0.001
% of energy as protein*	rho = -0.434	p = 0.04
% of energy as fat*	rho = -0.479	p = 0.022
% of energy as MUFA*	rho = -0.415	p = 0.05
% of energy as PUFA*	rho = -0.42	p = 0.047
% C24:0**	rho = 0.632	p = 0.002
% C20:4n-6**	rho = -0.444	p = 0.035
% C22:5n-3**	rho = 0.702	p = <0.001

Table D22: Variables showing a statistically significant association with % of energy as protein

Variable correlating with % of energy as protein*	Pearson's/Spearman's correlation coefficient	Level of significance
Heart rate (bpm)	r = 0.611	p = 0.002
Waist:hip ratio	r = 0.434	p = 0.038
Energy (kcal)*	rho = -0.521	p = 0.012
Carbohydrates (g)*	rho = -0.49	p = 0.018
Total fat (g)*	r = -0.635	p = 0.001
SFA (g)*	r = -0.613	p = 0.002
% of energy as carbohydrate*	rho = -0.434	p = 0.04
% C16:1n-7**	rho = 0.434	p = 0.04
% C18:1n-7**	r = 0.564	p = 0.005
Total % MUFA**	r = 0.417	p = 0.048
AUC of QRS (mm^2)	r = -0.492	p = 0.017
R wave amplitude (mV)	r = -0.468	p = 0.024

Table D23: Variables showing a statistically significant association with % of energy as fat

Variable correlating with % of energy as fat*	Pearson's/Spearman's correlation coefficient	Level of significance
Total fat (g)*	rho = 0.57	p = 0.005
SFA (g)*	rho = 0.396	p = 0.062
MUFA (g)*	rho = 0.421	p = 0.047
PUFA (g)*	rho = 0.596	p = 0.003
% of energy as carbohydrate*	rho = -0.479	p = 0.022
% of energy as SFA*	rho = 0.553	p = 0.007
% of energy as MUFA*	rho = 0.535	p = 0.001
% of energy as PUFA*	rho = 0.606	p = 0.003
% C16:1n-7**	rho = -0.456	p = 0.03
% C18:1n-7**	rho = -0.563	p = 0.006
% C18:2n-6**	rho = 0.427	p = 0.043
QRS duration (ms)	rho = 0.468	p = 0.024

Table D24: Variables showing a statistically significant association with % of energy as SFA

Variable correlating with % of energy as SFA*	Pearson's/Spearman's correlation coefficient	Level of significance
Haematocrit (%)	rho = 0.512	p = 0.012
SFA (g)*	rho = 0.651	p = 0.001
% of energy as fat*	rho = 0.553	p = 0.007
QRS duration (ms)	rho = 0.446	p = 0.033

Table D25: Variables showing a statistically significant association with % of energy as MUFA

Variable correlating with % of energy as MUFA*	Pearson's/Spearman's correlation coefficient	Level of significance
Carbohydrates (g)*	r = -0.497	p = 0.016
MUFA (g)*	rho = 0.649	p = 0.001
PUFA (g)*	rho = 0.632	p = 0.002
n-3 (g)*	r = 0.505	p = 0.014
% of energy as carbohydrate*	rho = -0.415	p = 0.05
% of energy as fat*	rho = 0.535	p = 0.01
% of energy as PUFA*	r = 0.786	p = <0.001
% of energy as TFA*	r = 0.467	p = 0.025
Total % MUFA**	r = -0.435	p = 0.038
% C18:2n-6**	rho = 0.469	p = 0.025
Total % n-6**	r = 0.525	p = 0.01

Table D26: Variables showing a statistically significant association with % of energy as PUFA

Variable correlating with % of energy as PUFA*	Pearson's/Spearman's correlation coefficient	Level of significance
Carbohydrate (g)*	r = -0.424	p = 0.044
MUFA (g)*	r = 0.611	p = 0.002
PUFA (g)*	rho = 0.834	p = <0.001
n-6 (g)*	rho = 0.65	p = 0.001
n-3 (g)*	rho = 0.507	p = 0.014
% of energy as carbohydrate*	rho = -0.42	p = 0.047
% or energy as fat*	rho = 0.606	p = 0.003
% of energy as MUFA*	r = 0.786	p = <0.001
% C18:2n-6**	r = 0.495	p = 0.016

Table D27: Variables showing a statistically significant association with % of energy as TFA

Variable correlating with % of energy as TFA*	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	rho = -0.499	p = 0.018
Hip circumference (cm)	rho = -0.434	p = 0.039
MUFA (g)*	r = 0.488	p = 0.018
TFA (g)*	rho = 0.816	p = <0.001
% of energy as MUFA**	r = 0.467	p = 0.025

Table D28: Variables showing a statistically significant association with weight

Variable correlating with weight (kg)	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = 0.886	p = <0.001
Hip circumference (cm)	r = 0.951	p = <0.001
Body fat %	r = 0.846	p = <0.001
BMI	rho = 0.906	P = <0.001
TFA (g)*	r = -0.473	p = 0.026
% of energy as TFA*	rho = -0.499	p = 0.018
QRS duration (ms)	r = 0.427	p = 0.047
PR interval (ms)	r = 0.475	p = 0.026
P wave duration (mV)	r = 0.43	p = 0.046

Table D29: Variables showing a statistically significant association with waist circumference

Variable correlating with waist circumference (cm)	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.886	p = < 0.001
Hip circumference (cm)	r = 0.857	p = < 0.001
Body fat %	r = 0.849	p = < 0.001
BMI	rho = 0.916	p = < 0.001
Waist:hip ratio	r = 0.539	p = 0.008
% C16:1n-7**	rho = 0.468	p = 0.024
% C18:1n-9**	r = 0.428	p = 0.041
Total % MUFA**	r = 0.442	p = 0.035
% C22:5n-3**	rho = -0.445	p = 0.033
% C22:6n-3**	r = -0.477	p = 0.021
% n-3**	r = -0.535	p = 0.008
QRS duration (ms)	r = 0.428	p = 0.042

Table D30: Variables showing a statistically significant association with hip circumference

Variable correlating with hip circumference (cm)	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.951	p = <0.001
Body fat %	r = 0.844	p = <0.001
BMI	r = 0.864	p = <0.001
TFA (g)*	r = -0.433	p = 0.039
% of energy as TFA*	rho = -0.434	p = 0.039
% C22:6n-3**	r = -0.433	p = 0.039
Total % n-3**	r = -0.424	p = 0.044
QRS duration (ms)	r = 0.492	p = 0.017
PR interval (ms)	r = 0.437	p = 0.037
P wave duration (ms)	rho = 0.467	p = 0.025

Table D31: Variables showing a statistically significant association with waist:hip ratio

Variable correlating with waist:hip ratio	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = 0.539	p = 0.008
% of energy as protein	r = 0.434	p = 0.038
% C16:1n-7**	rho = 0.498	p = 0.017
% C18:1n-9**	rho = 0.683	p = <0.001
Total % MUFA **	rho = 0.581	p = 0.004
Total % n-6**	r = -0.473	p = 0.023

Table D32: Variables showing a statistically significant association with body fat

Variable correlating with body fat %	Pearson's /Spearman's correlation coefficient	Level of significance
Weight (kg)	r = 0.846	p = <0.001
Waist circumference (cm)	r = 0.849	p = <0.001
Hip circumference (cm)	r = 0.844	p = <0.001
BMI	r = 0.900	p = <0.001
TFA (g)*	r = -0.448	p = 0.032
% C16:1n-7**	r = 0.488	p = 0.018

Table D33: Variables showing a statistically significant association with haematocrit

Variable correlating with haematocrit (%)	Pearson's/Spearman's correlation coefficient	Level of significance
n-3 (g)*	rho = -0.439	p = 0.036
n-6:n-3 ratio*	rho = 0.519	p = 0.011
% of energy as SFA*	rho = 0.512	p = 0.012
% C20:3n-3	r = 0.427	p = 0.042

Table D34: Variables showing a statistically significant association with BMI

Variable correlating with BMI	Pearson's/Spearman's correlation coefficient	Level of significance
Weight (kg)	rho = 0.906	p = <0.001
Waist circumference (cm)	rho = 0.916	p = <0.001
Hip circumference (cm)	r = 0.864	p = <0.001
Body fat %	r = 0.900	p = <0.001
TFA (g)*	rho = -0.424	p = 0.044
% of energy as carbohydrate	rho = -0.421	p = 0.046
% C22:6n-3**	r = -0.44	p = 0.036
Total % n-3**	r = -0.456	p = 0.029
QRS duration (ms)	rho = 0.418	p = 0.047

Table D35: Variables showing a statistically significant association with heart rate

Variable correlating with heart rate (bpm)	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = 0.558	p = 0.006
Total fat (g)*	r = -0.453	p = 0.03
% of energy as protein	r = 0.611	p = 0.002
% C16:0**	rho = 0.429	p = 0.041
% C16:1n-7**	rho = 0.558	p = 0.006
% C18:1n-9**	r = 0.475	p = 0.022
% C18:1n-7	r = 0.587	p = 0.003
% C22:1n-9**	rho = 0.445	p = 0.033
Total % MUFA**	r = 0.558	p = 0.006

Table D36: Variables showing a statistically significant association with SBP

Variable correlating with SBP (mmHg)	Pearson's/Spearman's correlation coefficient	Level of significance
Heart rate (bpm)	r = 0.558	p = 0.006
DBP (mmHg)	r = 0.647	p = <0.001
MAP (mmHg)	r = 0.857	p = <0.001
% C14:0**	r = 0.51	p = 0.013
% C18:0**	rho = -0.456	p = 0.029
% C20:0**	r = -0.477	p = 0.021
% C16:1n-7**	rho = 0.533	p = 0.009
% C24:1n-9**	r = -0463	p = 0.026
% DMA16**	r = -0.472	p = 0.023
% DMA18B**	r = -0.585	p = 0.003

Table D37: Variables showing a statistically significant association with DBP

Variable correlating with DBP (mmHg)	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = 0.647	p = <0.001
MAP (mmHg)	r = 0.947	p = <0.001
% C14:0**	rho = 0.633	p = <0.001
% C18:0**	rho = -0.651	p = <0.001
% C20:0**	r = -0.583	p = 0.004
% C22:0**	r = -0.442	p = 0.035
% C24:0**	r = -0.709	p = <0.001
% C16:1n-7**	rho = 0.512	p = 0.013
% C24:1n-9**	r = -0.707	p = <0.001
% DMA18A**	r = -0.487	p = 0.018
% DMA18B**	r = -0.779	p = <0.001

Table D38: Variables showing a statistically significant association with MAP

Variable correlating with MAP (mmHg)	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = 0.857	p = <0.001
DBP (mmHg)	r = 0.947	p = <0.001
% C14:0**	rho = 0.66	p = <0.001
% C18:0**	rho = -0.605	p = 0.002
% C20:0**	r = -0.595	p = 0.003
% C22:0**	r = -0.429	p = 0.041
% C24:0**	r = -0.643	p = <0.001
% C16:1n-7**	rho = 0.586	p = 0.003
% C24:1n-9**	r = -0.672	p = <0.001
% DMA16**	r = -0.479	p = 0.021
% DMA18A**	r = -0.515	p = 0.012
% DMA18B**	r = -0.773	p = <0.001

Table D39: Variables showing a statistically significant association with C14:0

Variable correlating with % C14:0**	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = 0.51	p = 0.013
DBP (mmHg)	rho = 0.633	p = <0.001
MAP (mmHg)	rho = 0.66	p = <0.001
% C18:0**	rho = -0.741	p = <0.001
% C20:0**	rho = -0.532	p = 0.009
% C22:0**	rho = -0.544	p = 0.008
% C24:0**	r = -0.496	p = 0.024
% C16:1n-7**	rho = 0.515	p = 0.013
% C24:1n-9**	rho = -0.534	p = 0.01
% DMA16**	rho = -0.614	p = 0.002
% DMA18A**	rho = -0.679	p = <0.001
% DMA18B**	rho = -0.564	p = 0.006

Table D40: Variables showing a statistically significant association with C16:0

Variable correlating with % C16:0**	Pearson's/Spearman's correlation coefficient	Level of significance
Heart rate (bpm)	rho = 0.429	p = 0.041
Total fat (g)*	rho = -0.499	p = 0.016
SFA (g)*	rho = -0.549	p = 0.007
% C24:0**	r = -0.487	p = 0.018
Total % SFA**	r = 0.664	p = <0.001
% C16:1n-7**	r = 0.742	p = <0.001
% C18:1n-9**	rho = 0.463	p = 0.027
% C24:1n-9**	r = -0.517	p = 0.012
% C18:2n-6**	rho = -0.701	p = <0.001
% C20:4n-6**	r = -0.487	p = 0.018
Total % n-6**	rho = -0.789	p = <0.001
Total % n-3**	r = -0.514	p = 0.012
% DMA18B**	r = -0.484	p = 0.019

Table D41: Variables showing a statistically significant association with C18:0

Variable correlating with % C18:0**	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	rho = -0.456	p = 0.029
DBP (mmHg)	rho = -0.651	p = <0.001
MAP (mmHg)	rho = -0.605	p = 0.002
% C14:0**	rho = -0.741	p = <0.001
% C20:0**	rho = 0.692	p = <0.001
% C22:0**	rho = 0.647	p = 0.001
% C24:0**	rho = 0.667	p = <0.001
Total % SFA**	rho = 0.623	p = 0.002
% C24:1n-9**	rho = 0.614	p = 0.002
% C20:2n-6**	rho = 0.423	p = 0.044
% DMA16**	rho = 0.424	p = 0.044
% DMA18A**	rho = 0.524	p = 0.011
% DMA18B**	rho = 0.682	p = <0.001

Table D42: Variables showing a statistically significant association with C20:0

Variable correlating with % C20:0**	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = -0.477	p = 0.021
DBP (mmHg)	r = -0.583	p = 0.004
MAP (mmHg)	r = -0.595	p = 0.003
% C14:0**	rho = -0.532	p = 0.009
% C18:0**	rho = 0.692	p = <0.001
% C22:0**	r = 0.567	p = 0.005
% C24:0**	rho = 0.464	p = 0.026
% DMA18B**	rho = 0.603	p = 0.002

Table D43: Variables showing a statistically significant association with C22:0

Variable correlating with % C22:0**	Pearson's/Spearman's correlation coefficient	Level of significance
DBP (mmHg)	r = -0.442	p = 0.035
MAP (mmHg)	r = -0.429	p = 0.041
% C14:0**	rho = -0.544	p = 0.008
% C18:0**	rho = 0.647	p = 0.001
% C20:0**	r = 0.567	p = 0.005
% C18:1n-7**	r = -0.52	p = 0.011
% C24:1n-9**	r = 0.438	p = 0.037
% C20:3n-6**	rho = -0.459	p = 0.029
% C20:3n-3**	rho = -0.506	p = 0.015
% DMA18A**	rho = 0.507	p = 0.015

Table D44: Variables showing a statistically significant association with C24:0

Variable correlating with % C24:0**	Pearson's/Spearman's correlation coefficient	Level of significance
Carbohydrate (g)*	r = 0.474	p = 0.022
% of energy as carbohydrate*	rho = 0.632	p = 0.002
DBP (mmHg)	r = -0.709	p = <0.001
MAP (mmHg)	r = -0.643	p = <0.001
% C14:0**	r = -0.469	p = 0.024
% C16:0**	r = -0.487	p = 0.018
% C18:0**	rho = 0.667	p = <0.001
% C20:0**	rho = 0.464	p = 0.026
% C16:1n-7**	r = -0.522	p = 0.011
% C24:1n-9**	r = 0.781	p = 0.001
% C20:4n-6**	r = 0.544	p = 0.007
% C18:3n-3**	rho = -0.417	p = 0.049
% C22:5n-3**	r = 0.562	p = 0.005
% DMA16**	r = 0.62	p = 0.002
% DMA18A**	r = 0.733	p = <0.001
% DMA18B**	r = 0.815	p = <0.001

Table D45: Variables showing a statistically significant association with SFA

Variable correlating with % of FA as SFA**	Pearson's/Spearman's correlation coefficient	Level of significance
n-6 (g)*	rho = -0.467	p = 0.026
Cholesterol (mg)*	r = -0.465	p = 0.025
% C16:0**	r = 0.664	p = <0.001
% C18:2n-6**	rho = -0.677	p = <0.001
% C20:2n-6**	r = 0.558	p = 0.006
Total % n-6**	rho = 0.758	p = <0.001
Total % n-3**	r = -0.566	p = 0.006
P wave amplitude (mV)	r = 0.547	p = 0.007
PR interval (ms)	r = 0.504	p = 0.014
P wave duration (ms)	r = 0.405	p = 0.055

Table D46: Variables showing a statistically significant association with C16:1n-7

Variable correlating with % C16:1n-7**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	rho = 0.468	p = 0.024
Waist:hip ratio	rho = 0.498	p = 0.017
Body fat %	r = 0.488	p = 0.018
Heart rate (bpm)	rho = 0.558	p = 0.006
SBP (mmHg)	rho = 0.533	p = 0.009
DBP (mmHg)	rho = 0.512	p = 0.013
MAP (mmHg)	rho = 0.586	p = 0.003
Total fat (g)*	r = -0.499	p = 0.015
% of energy as protein*	rho = 0.434	p = 0.04
% of energy as fat *	rho = -0.456	p = 0.03
% C14:0**	rho = 0.515	p = 0.013
% C16:0**	r = 0.742	p = <0.001
% C24:0**	r = -0.522	p = 0.011
% C18:1n-7**	rho = 0.656	p = <0.001
% C18:1n-9**	rho = 0.514	p = 0.013
% C24:1n-9**	rho = -0.424	p = 0.045
% C18:2n-6**	r = -0.46	p = 0.027
Total % MUFA**	rho = 0.721	p = <0.001
Total % n-6**	rho = -0.525	p = 0.011
% DMA18B**	rho = -0.576	p = 0.005

Table D47: Variables showing a statistically significant association with C18:1n-7

Variable correlating with % C18:1n-7**	Pearson's/Spearman's correlation coefficient	Level of significance
Heart rate (bpm)	r = 0.587	p = 0.003
Total fat (g)*	r = -0.591	p = 0.003
PUFA (g)*	r = -0.465	p = 0.025
% of energy as protein*	r = 0.564	p = 0.005
% of energy as fat*	rho = -0.563	p = 0.006
% C22:0**	r = -0.52	p = 0.011
% C16:1n-7**	rho = 0.656	p = <0.001
% C18:1n-9**	r = 0.478	p = 0.021
Total % MUFA**	r = 0.645	p = <0.001
% C20:3n-6**	rho = 0.416	p = 0.049
AUC of QRS (mm^2)	r = -0.46	p = 0.022
R wave amplitude (mV)	r = -0.44	p = 0.036

Table D48: Variables showing a statistically significant association with C18:1n-9

Variable correlating with % C18:1n-9**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = 0.428	p = 0.041
Waist:hip ratio	rho = 0.683	p = <0.001
Heart rate (bpm)	r = 0.475	p = 0.022
MUFA (g)*	r = -0.478	p = 0.021
% C16:0**	rho = 0.463	p = 0.027
% C16:1n-7**	rho = 0.514	p = 0.013
% C18:1n-7**	r = 0.478	p = 0.021
Total % MUFA**	r = 0.96	p = <0.001
% C18:2n-6**	rho = -0.536	p = 0.009
Total % n-6**	rho = -0.708	p = <0.001
% C20:5n-3**	rho = -0.479	p = 0.021
% C22:5n-3**	r = -0.508	p = 0.013
Total % n-3**	r = -0.484	p = 0.019

Table D49: Variables showing a statistically significant association with C20:1n-9

Variable correlating with % C20:1n-9**	Pearson's/Spearman's correlation coefficient	Level of significance
% C20:3n-3**	rho = 0.717	p = <0.001

Table D50: Variables showing a statistically significant association with C24:1n-9

Variable correlating with % C24:1n-9**	Pearson's/Spearman's correlation coefficient	Level of significance
% DMA16**	r = 0.604	p = 0.002
% DMA18A**	r = 0.533	p = 0.009
% DMA18B**	r = 0.807	p = <0.001

Table D51: Variables showing a statistically significant association with MUFA

Variable correlating with % of FA as MUFA**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = 0.442	p = 0.035
Waist:hip ratio	rho = 0.581	p = 0.004
Heart rate (bpm)	r = 0.558	p = 0.006
Total fat (g)*	r = -0.485	p = 0.019
MUFA (g)*	r = -0.559	p = 0.006
Cholesterol (mg)	r = -0.429	p = 0.042
% of energy as protein*	r = 0.417	p = 0.048
% of energy as MUFA*	r = -0.435	p = 0.038
% C16:1n-7**	rho = 0.721	p = <0.001
% C18:1n-7**	r = 0.645	p = <0.001
% C18:1n-9**	r = 0.96	p = <0.001
% C18:2n-6**	r = -0.652	p = <0.001
Total % n-6**	rho = -0.792	p = <0.001
Total % n-3**	r = 0.462	p = 0.027
R wave amplitude (mV)	r - -0.394	p = 0.063

Table D52: Variables showing a statistically significant association with C18:2n-6

Variable correlating with % C18:2n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
Total fat (g)*	r = 0.551	p = 0.006
MUFA (g)*	r = 0.679	p = <0.001
n-6 (g)*	rho = 0.444	p = 0.035
n-3 (g)*	rho = 0.484	p = 0.019
PUFA (g)*	rho = 0.666	p = <0.001
Cholesterol (mg)	rho = 0.639	p = <0.001
% of energy as fat*	rho = 0.427	p = 0.043
% of energy as MUFA*	rho = 0.469	p = 0.025
% of energy as PUFA*	r = 0.495	p = 0.016
% C16:0**	rho = -0.701	p = <0.001
Total % SFA**	rho = -0.677	p = <0.001
% C16:1n-7**	r = -0.46	p = 0.027
% C18:1n-9**	rho = -0.536	p = 0.009
Total % MUFA**	r = -0.652	p = <0.001
% C20:2n-6**	r = -0.445	p = 0.034
Total % n-6**	rho = 0.912	p = <0.001

Table D53: Variables showing a statistically significant association with C18:3n-6

Variable correlating with % C18:3n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
% C20:5n-3**	rho = 0.655	p = <0.001
% DMA18A**	rho = -0.527	p = 0.011

Table D54: Variables showing a statistically significant association with C20:2n-6

Variable correlating with % C20:2n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
% C18:0**	rho = 0.423	p = 0.044
Total % SFA**	r = 0.558	p = 0.006
% C18:2n-6**	r = -0.445	p = 0.034
Total % n-6**	r = -0.535	p = 0.009
% C20:5n-3**	rho = -0.458	p = 0.028
Total % n-3**	r = -0.498	p = 0.016
QT duration (ms)	r = -0.506	p = 0.014
ARP (ms)	r = -0.46	p = 0.027

Table D55: Variables showing a statistically significant association with C20:3n-6

Variable correlating with % C20:3n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
Carbohydrates (g)*	r = 0.558	p = 0.006
% C22:0**	rho = -0.459	p = 0.029
% C18:1n-7**	rho = 0.416	p = 0.049
P wave duration (ms)	rho = -0.439	p = 0.036

Table D56: Variables showing a statistically significant association with C20:4n-6

Variable correlating with % C20:4n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
% of energy as carbohydrate*	rho = -0.444	p = 0.035
% C16:0**	r = -0.487	p = 0.018
% C24:0**	r = 0.544	p = 0.007
% C18:3n-3**	rho = -0.446	p = 0.034
% C20:3n-3**	rho = 0.458	p = 0.029
% C22:5n-3**	r = 0.672	p = <0.001
% C22:6n-3**	r = 0.437	p = 0.037
Total % n-3**	r = 0.546	p = 0.007
% DMA16**	r = 0.506	p = 0.014
% DMA18A**	r = 0.459	p = 0.028

Table D57: Variables showing a statistically significant association with n-6

Variable correlating with % of FA as n-6**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist:hip ratio	r = -0.473	p = 0.023
Total fat (g)*	r = 0.551	p = 0.006
SFA (g)*	rho = 0.452	p = 0.032
MUFA (g) *	r = 0.64	p = <0.001
PUFA (g)*	r = 0.439	p = 0.036
% of energy as MUFA*	r = 0.525	p = 0.01
% C16:0**	rho = -0.789	p = <0.001
Total % SFA**	rho = 0.758	p = <0.001
% C16:1n-7**	rho = -0.525	p = 0.011
% C18:1n-9**	rho = -0.708	p = <0.001
Total % MUFA**	rho = -0.792	p = <0.001
% C18:2n-6**	rho = 0.912	p = <0.001
% C20:2n-6**	r = -0.535	p = 0.009
% C22:6n-3**	r = 0.456	p = 0.029
Total % n-3**	r = 0.577	p = 0.004

Table D58: Variables showing a statistically significant association with C18:3n-3

Variable correlating with % C18:3n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
n-3 (g)*	rho = 0.539	p = 0.008
% C24:0**	rho = -0.417	p = 0.049
% C20:4n-6**	rho = -0.446	P = 0.034
% DMA16**	rho = -0.571	p = 0.004
% DMA18A**	rho = -0.456	p = 0.03
% DMA18B**	rho = -0.421	p = 0.047

Table D59: Variables showing a statistically significant association with C20:3n-3

Variable correlating with % C20:3n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
Haematocrit (%)	r = 0.427	p = 0.042
% C22:0**	rho = -0.506	p = 0.015
%C20:1n-9**	rho = 0.717	p = <0.001
% C24:1n-9**	r = 0.569	p = 0.005
% C20:4n-6**	rho = 0.458	p = 0.029

Table D60: Variables showing a statistically significant association with C20:5n-3

Variable correlating with % C20:5n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
% C18:1n-9**	rho = -0.479	p = 0.009
% C18:3n-6**	rho = 0.655	p = <0.001
% C20:2n-6**	rho = -0.458	p = 0.028
Total % n-3**	rho = 0.471	p = 0.023
% DMA18A**	r = -0.419	p= 0.047

Table D61: Variables showing a statistically significant association with C22:5n-3

Variable correlating with % C22:5n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	rho = -0.445	p = 0.033
Carbohydrates (g)*	r = 0.445	p = 0.033
% of energy as carbohydrate*	rho = 0.702	p = <0.001
% C24:0**	r = 0.562	p = 0.005
% C18:1n-9**	r = -0.508	p = 0.013
Total % MUFA**	r = -0.451	p = 0.031
% C20:4n-6**	r = 0.672	p = <0.001
Total % n-3**	r = 0.484	p = 0.019
% DMA16**	r = 0.533	p = 0.009
% DMA18A**	rho = 0.533	p = 0.01

Table D62: Variables showing a statistically significant association with C22:6n-3

Variable correlating with % C22:6n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
Hip circumference (cm)	r = -0.433	p = 0.039
BMI	r = -0.44	p = 0.036
Total % SFA**	r = -0.47	p = 0.024
% C20:4n-6**	r = 0.437	p = 0.037
Total % n-6**	r = 0.456	p = 0.029
Total % n-3**	rho = 0.872	p = <0.001
n-6:n-3 ratio**	r = -0.712	p = <0.001
AUC of QRS (mm^2)	r = -0.463	p = 0.026

Table D63: Variables showing a statistically significant association with n-3

Variable correlating with % of FA as n-3**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = -0.535	p = 0.008
Hip circumference (cm)	r = -0.424	p = 0.044
BMI	r = -0.456	p = 0.029
% C16:0**	r = -0.514	p = 0.012
Total % SFA**	r = -0.566	p = 0.006
% C18:1n-9**	r = -0.484	p = 0.019
Total % MUFA**	r = 0.462	p = 0.027
% C20:2n-6**	r = -0.498	p = 0.016
% C20:4n-6**	r = 0.546	p = 0.007
Total % n-6**	r = 0.577	p = 0.004
% C20:5n-3**	rho = 0.471	p = 0.023
% C22:5n-3**	r = 0.484	p = 0.019
% C22:6n-3**	rho = 0.872	p = <0.001
n-6:n-3 ratio**	r = -0.731	p = <0.001

Table D64: Variables showing a statistically significant association with PUFA

Variable correlating with % of FA as PUFA**	Pearson's/Spearman's correlation coefficient	Level of significance
Waist circumference (cm)	r = -0.428	p = 0.041
Waist:hip ratio	r = -0.461	p = 0.027
Total fat (g)*	r = 0.474	p = 0.022
MUFA (g)*	r = 0.578	p = 0.004
Cholesterol (mg)*	rho = 0.567	p = 0.05
% C16:0**	rho = -0.769	p = <0.001
Total % SFA**	rho = -0.8	p = <0.001
% C16:1n-7**	rho = -0.528	p = 0.01
% C18:1n-9**	rho = -0.721	p = <0.001
Total % MUFA**	rho = -0.782	p = <0.001
% C18:2n-6**	rho = 0.865	p = <0.001
% C20:2n-6**	r = -0.572	p = 0.004
% C20:4n-6**	rho = 0.450	p = 0.033
Total % n-6**	r = 0.988	p = <0.001
% C22:6n-3**	r = 0.569	p = 0.005
Total % n-3**	r = 0.696	p = <0.001

Table D65: Variables showing a statistically significant association with n-6:n-3 ratio

Variable correlating with n-6:n-3 ratio**	Pearson's/Spearman's correlation coefficient	Level of significance
Haematocrit (%)	rho = 0.519	p = 0.011
Energy (kcal)*	r = 0.436	p = 0.038
Total fat (g)*	r = 0.498	p = 0.016
TFA (g)*	r = 0.452	p = 0.03
% C22:6n-3**	r = -0.712	p = <0.001
Total % n-3**	r = -0.731	p = <0.001
AUC of QRS (mm^2)	r = 0.576	p = 0.004
R wave amplitude (mV)	rho = 0.423	p = 0.045

Table D66: Variables showing a statistically significant association with DMA16

Variable correlating with % DMA16**	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = -0.472	p = 0.023
MAP (mmHg)	r = -0.479	p = 0.021
% C14:0**	rho = -0.614	p = 0.002
% C18:0**	rho = 0.424	p = 0.044
% C24:0**	r = 0.62	p = 0.002
% C24:1n-9**	r = 0.604	p = 0.002
% C20:4n-6**	r = 0.506	p = 0.014
% C18:3n-3**	rho = -0.571	p = 0.004
% C22:5n-3**	r = 0.533	p = 0.009
% DMA18A**	r = 0.74	p = <0.001
% DMA18B**	r = 0.56	p = 0.005

Table D67: Variables showing a statistically significant association with DMA18A

Variable correlating with % DMA18A**	Pearson's/Spearman's correlation coefficient	Level of significance
% C18:0**	rho = 0.524	p = 0.011
% C22:0**	rho = 0.507	p = 0.015
% C24:0**	r = 0.733	p = <0.001
% C24:1n-9**	r = 0.533	p = 0.009
% C18:3n-6**	rho = -0.527	p = 0.011
% C20:4n-6**	r = 0.459	p = 0.028
% C18:3n-3**	rho = -0.456	p = 0.03
% C20:5n-3**	r = -0.419	p = 0.047
% C22:5n-3**	rho = 0.533	p = 0.01
% DMA16**	r = 0.74	p = <0.001
% DMA18B**	rho = 0.601	p = 0.003

Table D68: Variables showing a statistically significant association with DMA18B

Variable correlating with % DMA18B**	Pearson's/Spearman's correlation coefficient	Level of significance
SBP (mmHg)	r = -0.585	p = 0.003
DBP (mmHg)	r = -0.779	p = <0.001
MAP (mmHg)	r = -0.773	p = <0.001
% C14:0**	rho = -0.564	p = 0.006
% C16:0**	r = -0.484	p = 0.019
% C18:0**	rho = 0.682	p = <0.001
% C20:0**	rho = 0.603	p = 0.002
% C24:0**	r = 0.815	p = <0.001
% C16:1n-7**	rho = -0.576	p = 0.005
% C24:1n-9**	r = 0.807	p = <0.001
% C18:3n-3**	rho = -0.421	p = 0.047
% DMA16**	r = 0.56	p = 0.005
% DMA18A**	rho = 0.601	0.003