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Olive oil, compared to a saturated dietary fat, has a protective role on atherosclerosis in niacin-treated mice with metabolic syndrome

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ABSTRACT

We aimed to investigate the impact of high-fat low-cholesterol diets rich in saturated fatty acids (HFLCD-SFAs), monounsaturated FAs (HFLCD-MUFAs) or MUFAs + omega-3 long-chain polyunsaturated FAs (HFLCD-PUFAs) in combination with niacin (NA) on atherosclerotic plaque characteristics in a mouse model ($Lep^{ob/ob}LDLR^{-/-}$) of metabolic syndrome (MetS). Compared to a low-fat low-cholesterol diet (LFLCD), HFLCDs increased body weight, triglycerides, insulin, pro-inflammatory cytokines, and circulating monocytes, contributing the HFLCD-SFAs to a predominance of a classical pro-inflammatory $Ly6C^{hi}$ population, whereas HFLCD-MUFAs and HFLCD-PUFAs to a non-classical patrolling $Ly6C^{lo}$ population. HFLCDs promoted atherosclerosis in the aortic roots of animals but the plaque size, collagen, and macrophage content were higher with the HFLCD-SFAs than with the HFLCD-MUFAs or HFLCD-PUFAs. Furthermore, HFLCD-SFAs promoted the intra-plaque accumulation of M1 macrophages, whereas HFLCD-MUFAs and HFLCD-PUFAs favoured the accumulation of M2 macrophages. These data suggest that dietary MUFAs had advantage over SFAs to prevent atherosclerotic events in the NA-treated MetS.

1. Introduction

Dyslipidaemia, insulin resistance, and obesity, the defining components of the metabolic syndrome (MetS), are well-known risk factors implicated in the aetiology and pathogenesis of certain cardiovascular diseases (CVDs) such as atherosclerosis, the main cause of CVD death in developed and some developing countries (Libby, 2012). Atherosclerosis is a systemic lipid-driven inflammatory condition associated with endothelial dysfunction that results in accumulation and subsequent oxidation of lipids in the vessel wall or plaque development. These abnormalities trigger inflammatory cell infiltration and macrophage foam cell formation leading to apoptosis and secondary necrosis and plaque advancement (Tabas, 2010). It is important to address the factors involved in the progression of

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atherosclerosis because advanced atherosclerotic lesions are prone to rupture, leading to disability or death. The monocyte–macrophage lineage is of fundamental interest in understanding atherosclerotic lesion progression (Ziegler-Heitbrock et al., 2010). Monocytes can be found into two distinct subsets based on the expression of specific surface markers. In mice, monocytes are divided based on Ly6C expression into inflammatory Ly6C high ($Ly6C^{hi}$) and patrolling Ly6C low ($Ly6C^{lo}$) monocytes (Auffray et al., 2007; Sunderkotter et al., 2004). CC-motif chemokine receptor 2 (CCR2) is expressed at high levels by inflammatory monocytes (Zlotnik & Yoshie, 2000), whereas CX_3C-motif chemokine receptor 1 (CX_3CR1) is more abundant on patrolling monocytes (Geissmann, Jung, & Littman, 2003; Woollard & Geissmann, 2010). Inflammatory monocytes are generally thought to be the most important subtype in early atherogenesis, being efficiently recruited to the lesion in a CCR2-dependent manner (Robbins et al., 2012; Soehnlein et al., 2013).

The atheroprotective effects of niacin (NA), also known as nicotinic acid or vitamin B3, were first described in the 1950s (Carlson, 2005; Montserrat-de la Paz et al., 2016a). According to a meta-analysis of 30 randomized controlled trials, NA potently reduces triglycerides (TGs) by 15–30%, total cholesterol (TC) by 5–15%, and LDL-cholesterol (LDL-C) by 5–20% and increases HDL-cholesterol (HDL-C) by 10–25% in plasma of patients with dyslipidaemia and/or hypercholesterolaemia (Birjmohun, Hutten, Kastelein, & Stroes, 2005). Additional to the pharmacology of NA, several translational studies have identified the differential role between saturated and unsaturated fats at cardiovascular level. Compared to saturated fatty acids (SFAs), the consumption of monounsaturated (MUFAs) and omega-3 long-chain polyunsaturated (PUFAs) fatty acids have beneficial effects on lowering blood lipids (Ortega et al., 2012; Vafeiadou et al., 2015) and inflammatory mediators (Naranjo et al., 2016; Teng, Chang, Chang, & Nesaretnam, 2014). However, the potential anti-atherogenic effects from a combination of dietary fatty acids and NA have not yet been defined. Therefore, the current study aimed to explore the influence of a pharmacological dose of NA and diets enriched in SFAs, MUFAs or MUFAs + omega-3 long-chain PUFAs on atherosclerosis in a mouse model ($Lep^{ob/ob}LDLR^{-/-}$) of MetS.

2. Materials and methods

2.1. Fatty acid composition of dietary fats

The fatty acid composition of dietary fats [cow's milk cream, rich in SFAs; refined olive oil, rich in MUFAs; and refined olive oil plus eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), rich in MUFAs and omega-3 long-chain PUFAs] was determined by the method described in EEC/796/2002 (Montserrat-de la Paz et al., 2016b), using a gas chromatography system (HP-5890, Hewlett-Packard, Palo-Alto, USA) equipped with flame ionization detector and a SP-2380 capillary column (Supelco, Bellefonte, USA, 30 m × 0.32 mm) packed with cyanopropyl siloxane (0.25 μm). The initial column temperature was 165 °C, which was held for 10 min, then programmed from 165 °C to 200 °C at 1.5 °C/min. Injector and detector temperatures were 250 °C, with the carrier gas H_2. The fatty acid composition of different dietary fats is detailed in Table 1.

Table 1 – Fatty acid composition of dietary fats.

Fatty acid	Cow's milk cream g/100 g of fatty acid	Refined olive oil	Refined olive oil plus EPA + DHA
10:0, capric	2.5 ± 0.1	–	–
12:0, lauric	3.1 ± 0.4	–	–
14:0, myristic	10.9 ± 0.9	–	–
16:0, palmitic	35.5 ± 0.8	20.4 ± 0.9	20.5 ± 0.6
16:1(n-7), palmitoleic	3.6 ± 0.3	1.0 ± 0.2	0.8 ± 0.1
18:0, stearic	11.5 ± 0.8	5.7 ± 0.1	4.5 ± 0.4
18:1(n-9), oleic	25.3 ± 0.7	61.9 ± 1.2	61.5 ± 1.0
18:2(n-6), linoleic	4.3 ± 0.8	8.0 ± 0.7	8.0 ± 0.5
18:3(n-3), α-linolenic	0.4 ± 0.1	1.0 ± 0.1	0.9 ± 0.0
20:5(n-3), eicosapentaenoic	–	–	0.9 ± 0.1
22:6(n-3), docosahexaenoic	–	–	0.7 ± 0.1
Others	3.0 ± 1.7	2.1 ± 1.1	2.0 ± 0.9
SFAs	63.5 ± 1.9[a]	26.1 ± 1.0[b]	25.0 ± 0.9[b]
MUFAs	28.9 ± 0.8[b]	62.8 ± 1.4[a]	62.4 ± 1.0[a]
PUFAs	4.7 ± 0.8[c]	9.0 ± 0.7[b]	10.6 ± 0.7[a]

Values are expressed as the mean ± SD ($n = 3$) and those marked with different lowercase letters in the same row are statistically different ($P < 0.05$).

2.2. Animal diets and experimental design

Male $Lep^{ob/ob}LDLR^{-/-}$ mice bred onto a C57BL/6J background (B6.Cg-Lepob Ldlrtm1Her/J, The Jackson Laboratory, Bar Harbor, ME, USA) was used for the study. These mice are obese and develop plasma lipid alterations that closely reflect MetS-related hyperlipidaemia (Kennedy, Ellacott, King, & Hasty, 2010; Montserrat-de la Paz et al., 2016c). All diets were prepared by Panlab Laboratoires (SAFE, Augy, France) and presented as pellets to the animals. Mice received one of the following diets for 8 weeks: a standard normal-fat diet (low-fat low-cholesterol diet, LFLCD) containing 3% energy as fat, used as control, or high-fat low-cholesterol diets (HFLCDs), which contained 24% energy as fat. All the diets were based on the standard rodent diet A04-10, containing 0.01% cholesterol, 20 mg/kg BHT, and 3% binder. Three different HFLCDs were prepared by replacing the fat source from A04-10 diet by cow's milk cream (21% energy) (HFLCD-SFAs), refined olive oil (21% energy) (HFLCD-MUFAs) or refined olive oil (20% energy) plus EPA+DHA in the form of ethyl esters (1% energy) (HFLCD-PUFAs). The cow's milk cream provided an additional amount of 0.006% by weight. All the diets contained equal proportion of protein (19.5% energy) and carbohydrate was used to adjust the total energy content.

After weaning, mice were randomly allocated into 4 groups (n = 10 per group) as follows: (1) group that received LFLCD; (2) group that received HFLCD-SFAs; (3) group that received HFLCD-MUFAs; and (4) group that received HFLCD-PUFAs. The four groups received NA (1%, w/v; Twinlab, UT, USA) in the drinking water. Body weight, food, and water intake were daily evaluated. Sacrifice of all animals was carried out within the animal facilities (Instituto de Biomedicina de Sevilla, IBiS) at the beginning of the light cycle and after 10 h of food deprivation. Animals were euthanized with an overdose of pentobarbital (1:10 in PBS, 150 mg/kg body weight). Cardiac