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The effects of fenofibrate on metabolic and vascular changes induced by chocolate-supplemented diet in the rat

Ebrahim K. Naderali *, Sameer Fatani

Neuroendocrine & Obesity Biology Unit, Department of Medicine, University of Liverpool, UCD, Daulby Street, Liverpool L69 3GA, UK

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Abstract

Current evidences suggest that diet per se plays an important role in genesis of metabolic and vascular function abnormalities. We have investigated the effects of addition of a high-fat diet (chocolate) in the presence or absence of short-term (7 days) administration of fenofibrate on metabolic and vascular changes in adult male Wistar rats. Despite similarities in total body weight in all groups, compared with control fed groups, chocolate-supplemented animals had significantly higher plasma triacylglyceride and non-esterified fatty acids and leptin (for all, $P<0.01$), but not glucose or insulin levels. Fenofibrate treatment corrected metabolic changes. In the mesenteric arteries, responses to KCl and noradrenaline were significantly (for both, $P<0.01$) higher in chocolate-supplemented group. Furthermore, vasorelaxant responses to carbamylcholine, but not to sodium nitroprusside, were significantly ($P<0.01$) reduced in the chocolate-supplemented group. Although fenofibrate failed to improve noradrenaline and KCl responses, it was effective in improving carbamylcholine-induced vasorelaxation. These data suggest that high-fat diet has a profound effect on plasma lipid profile and vascular function. Furthermore, fenofibrate treatment may ameliorate high-fat diet effects on vascular function and metabolic changes.
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Keywords: High-fat diet; Vascular contractility; Endothelial dysfunction; Fenofibrate; Hypertriglycerideamia

1. Introduction

Acceleration in the rate of diet-induced obesity throughout the world is becoming a major health problem. Excess energy intake in the form of refined carbohydrates and fatty acids is one of the main causes of human obesity. Obesity is a major risk factor for cardiovascular disorders, which include hypertension, atherosclerosis and ultimately myocardial infarction (Andres, 1980; Lakka et al., 2002). Arterial function and structural abnormalities which include alterations of the release of vasoactive substances from endothelial cells as well as changes in arterial wall elasticity have been seen in obese human subjects and certain animal models that reflect some aspects of human obesity (Cowan et al., 1991; Dobrian et al., 2000). Moreover, human and animal studies have shown various degrees of endothelial dysfunction in obesity, namely a reduction to vasorelaxant's response (Abram, 1997, Naderali et al., 2001; Perticone et al., 2001; Steinberg et al., 1996).

Increasing reports suggest that diet and its variant component play important roles in arterial function. Studies have shown that consumption of a specific diet of high fat or low protein causes endothelial abnormalities in humans and animals, while a low fat diet has a protective effect on vascular reactivity (Vogel et al., 1997; Verhamme et al., 2002; Naderali et al., 2004). Moreover, pharmacological lowering of plasma lipids by fenofibrate, retards atheroma developments (Renier et al., 2000), indicating a need to control circulating plasma lipids. Therefore, the aim of this study was to investigate the effects of supplementation of a high-fat diet in the presence or absence of short-term (7 days) administration of fenofibrate on metabolic changes and vascular function.

* Corresponding author. Tel.: +44 151 706 4087; fax: +44 151 706 5626.
E-mail address: naderali@liverpool.ac.uk (E.K. Naderali).

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2. Materials and methods

2.1. Animals and experimental protocol

Adult male Wistar rats ($n=21$) were randomized and assigned to two groups which were fed on either a standard pelletted laboratory chow (CRM Biosure, Cambridge, UK; which provided 60% of energy as carbohydrate, 30% as protein and 10% as fat) throughout ($n=7$; control), or given chow plus Galaxy smooth and creamy milk chocolate (Mars) (which provided 42.2% of energy as carbohydrate, 5.1% as protein and 52.7% as fat) ($n=14$; chocolate-supplemented). After 14 weeks, half of the chocolate-supplemented group was given fenofibrate (50 mg/kg/day) and the remainder were given vehicle (1% carboxymethyl cellulose at 1 ml/kg body weight; Sigma, UK) by oral gavage daily for 7 days, before being sacrificed. All animals had free access to their respective diets and water, and were housed in groups of 2 under controlled environmental conditions (19–22 °C; 30–40% humidity) and a 12-h light/dark cycle (lights on at 07:00 h). Daily energy intake was determined in all three groups for the last 7 days of the study.

The rats were killed by CO_2 inhalation, and the epididymal and perirenal fat pads and the gastrocnemius and cardiac muscles were dissected and weighed. Blood was collected for haematocrite studies and for the measurement of glucose, insulin, leptin, non-esterified fatty acids and triacylglycerol. Plasma glucose concentration was determined by the glucose oxidase method, while non-esterified fatty acids and triacylglycerol concentrations were determined by commercial diagnostic kits (Boehringer Mannheim, U.K and Sigma, U.K, respectively). Insulin and leptin concentrations were measured by radioimmunoassay (RIA) kits (Pharmacia/Upjohn Diagnostics, U.K. and Linco Research, U.K, respectively).

2.2. Assessment of vascular function

Six third-order mesenteric arteries from each animal (150–180 μM diameter, 2 mm lengths) were carefully dissected from each animal and mounted on two 40-μm diameter stainless-steel wires in an automated myograph (Cambustion, Cambridge, UK). Pairs of arteries were incubated in a 5-ml organ bath containing physiological salt solution (PSS; composition [in mM]: NaCl 119, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.17, $NaHCO_3$ 25, KH_2PO_4 1.18, EDTA 0.026 and glucose 5.5) gassed with 95% O_2 and 5% CO_2 at 37 °C.

After 30 min equilibration, the length–tension characteristics for each vessel were determined (Naderali et al., 2000). Arteries were allowed a further 30 min to equilibrate before being depolarised twice with high-potassium physiological salt solution (KPSS) in which NaCl in normal PSS was replaced by an equimolar concentration of KCl (125 mM). Cumulative concentration–response curves to either KCl (10–125 mM) or noradrenaline (0.5–6 μM) were then carried out. Any vessel failing to reach its predetermined target tension in response to KCl (125 mM) was discarded. We have also measured the effects of selected endothelium-dependent vasodilator, carbamylcholine, and endothelium-independent vasodilator, sodium nitroprusside on arteries preconstricted with 8 μM noradrenaline.

2.3. Reagents

Noradrenaline, carbamylcholine, sodium nitroprusside, and fenofibrate were all obtained from Sigma Chemicals. All solutions were prepared freshly in distilled water prior to use.

2.4. Data interpretation and statistical analyses

KCl- and noradrenaline-induced vasoconstriction responses were expressed as absolute force generated. Relaxation in response to carbamylcholine and sodium nitroprusside were calculated as the percentage reduction from the maximal tension generated in response to a supramaximal concentration of noradrenaline (8 μM). An average response for all the vessels from a given animal was determined before group analysis. Data are presented as mean±S.E.M. Statistical significance was tested using repeated-measures analysis of variance (ANOVA) or the Mann–Whitney test, as appropriate. Differences were considered statistically significant at values of $P<0.05$.

3. Results

3.1. Metabolic data

Supplementation of chocolate did not alter total body weight gain. In fact throughout the experiment there were no significant differences in total body weight at any given time

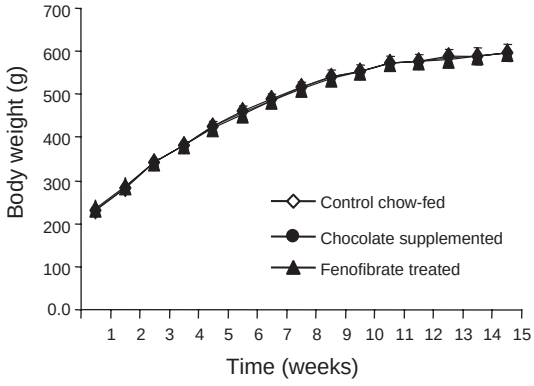

Fig. 1. The effect of chocolate supplementation and fenofibrate treatment on total body weight in the rat. Control animals were fed standard chow while chocolate-supplemented groups was given chow plus chocolate (Galaxy) for 15 weeks. Fenofibrate-treated rats were given fenofibrate (50 mg/kg/day) daily for 7 days. At the end of 15 weeks, there were no significant changes in total body weight among the three groups. In fact weight increase in all three groups were superimposable. Data are expressed as mean±S.E.M.

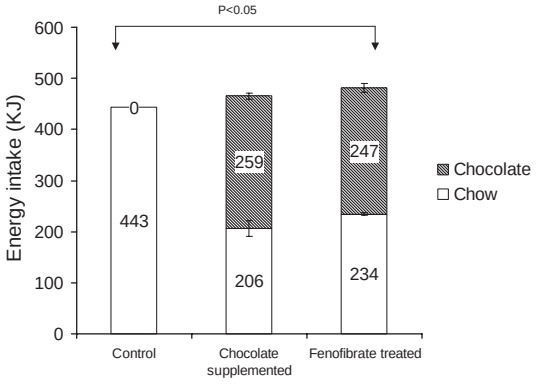

Fig. 2. Cumulative weekly energy intake of animals from all three groups during fenofibrate (50 mg/kg/day) treatment period. The increase in total energy intake of animals on chocolate supplementation was not statistically significant ($P=0.346$), however fenofibrate treatment further increased energy intake, which was significantly ($P<0.05$) higher than chow-fed controls.

between chow-fed control and chocolate-supplemented test groups (Fig. 1). Furthermore, the epididymal and perirenal fat pad masses as well as gastrocnemius muscle mass were comparable in all three groups, indicating the absence of any obesity. Analysis of food intake during the last 7 days showed that compared with control chow-fed rats, increase in total weekly energy intake of chocolate-supplemented group was insignificant (Fig. 2). However, fenofibrate-treated rats had significantly ($P<0.05$) higher energy intake than control chow-fed groups (Fig. 2). Nonetheless, this increase in energy intake did not translate to a significant increase in total body weight (Table 1).

While supplementation of chocolate had no significant effect on cardiac muscle mass or haematocrit values, fenofibrate-treated animals have significantly ($P<0.01$) higher cardiac muscle mass and reduced haematocrit values (Table 1). At the end of the experiment, fasting plasma glucose, insulin levels were comparable between control chow-fed and chocolate-supplemented groups, however, fenofibrate-treated animals had significantly ($P<0.01$) lower glucose and insulin levels than control counterparts. Furthermore, chocolate-supplemented animals had significantly higher fasting concentrations of plasma leptin, triacylglycerol and non-esterified fatty acids than chow-fed controls. These changes were almost completely reversed by fenofibrate treatment (Table 1).

Table 1
Physiological and metabolic characteristics of the 3 experimental groups

	Chow-fed controls ($n=7$)	Chocolate-supplemented ($n=7$)	Fenofibrate-treated ($n=7$)
Body weight (g)	598±23	606±19	611±21
Epididymal fat pad mass (g)	6.56±0.80	7.20±0.59	6.56±0.64
Perirenal fat pad mass (g)	6.20±0.94	7.24±0.66	6.69±0.84
Gastrocnemius muscle mass (g)	3.27±0.15	3.06±0.11	3.16±0.11
Heart muscle mass (g)	1.57±0.09	1.54±0.05	1.70±0.08 [a, b]
Haematocrit	49.1±0.6	47.5±0.8	46.4±0.5 [a]
Plasma glucose (mM)	11.3±0.9	13.5±1.0	9.3±0.4 [a, b]
Plasma insulin (μU/ml)	1.52±.38	1.31±0.33	1.12±0.21 [a]
Plasma leptin (ng/ml)	15.1±2.2	21.6±4.0 [a]	14.2±6.9 [b]
Plasma triglycerides (mM)	1.50±0.06	1.96±0.12 [a]	0.96±0.08 [a, b]
Plasma NEFA (mM)	0.28±0.02	0.39±0.02 [a]	0.23±0.01 [b]

Data are mean±SEM.
[a] $P<0.01$ vs controls.
[b] $P<0.01$ vs chocolate-supplemented.

3.2. Agonist-induced contractile responses

KCl (10–125 mM)- and noradrenaline (0.5–6 μM)-induced vasoconstriction in arteries from all three groups

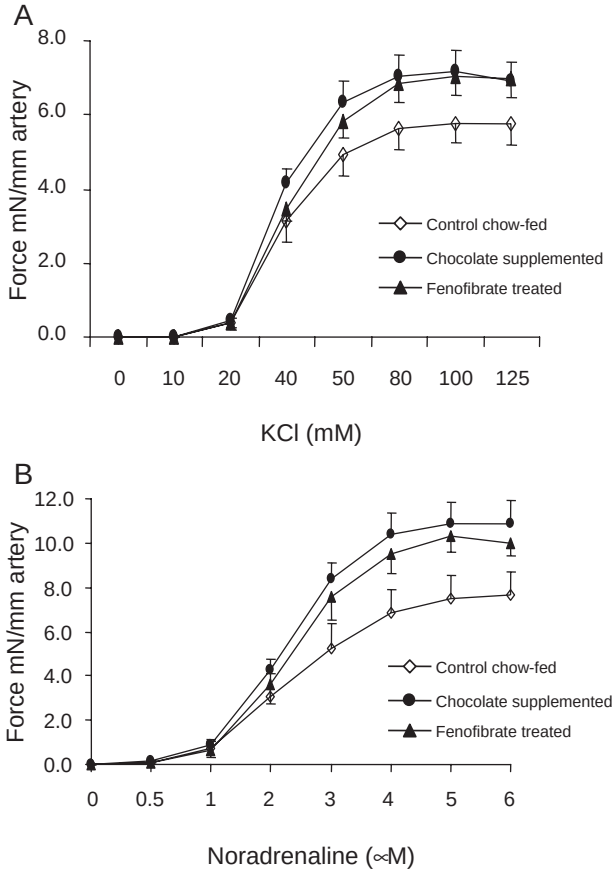

Fig. 3. Vasoconstriction responses to cumulative concentration of (A) KCl (10–125 mM) and (B) noradrenaline (0.5–6 μM) in arteries from chow-fed control, chocolate-supplemented and fenofibrate-treated animals. Both KCl and noradrenaline-induced contractions were significantly (ANOVA, $P<0.01$) augmented in arteries from chocolate-supplemented animals. Data represent mean±S.E.M.

produced a characteristic sigmoid relationship. The contractile responses to KCl and noradrenaline in arteries from chocolate- supplemented animals (with or without fenofibrate treatment) were significantly ($P<0.001$) increased in comparison to control groups (Fig. 2). The maximal KCl-induced contractions were 5.78±0.54 control, 7.14±0.57 chocolate-supplemented, and 7.07±0.52 mN/mm artery fenofibrate-treated, respectively (Fig. 3A). A similar picture was also seen with noradrenaline-induced vasoconstriction giving maximal contraction of 7.68±1.02 control, 10.91±0.96 supplemented, and 10.34±0.66 mN/mm artery fenofibrate-treated, respectively (Fig. 3B).

3.3. Endothelium-dependent and -independent vasorelaxation

Noradrenaline preconstricted arteries from chow-fed rats demonstrated progressive relaxation to cumulative addition of carbamylcholine (10 nM–100 μM), achieving a maximum of 87±2% at a carbamylcholine concentration of 100 μM. Arteries from test and fenofibrate-treated groups that were similarly exposed to carbamylcholine displayed similar responses with maximal relaxation of 85±6% and 92±2% (Fig. 4A). However, arteries from chocolate-supplemented rats had significantly ($P<0.01$) higher EC_{50} than control chow-fed animals (EC_{50}: 1.86±0.02 chocolate-supplemented, vs EC_{50}: 0.28±0.01 μM chow-fed). Fenofibrate treatment significantly ($P<0.05$) improved EC_{50} compared with chocolate-supplemented group (EC_{50}: 0.68±0.01 μM fenofibrate treatment vs EC_{50}: 1.86±0.02 μM chocolate-supplemented).

Endothelium-independent vasodilator (sodium nitroprusside; 10 nM–100 μM) also induced concentration dependent vasorelaxation of arteries from all three groups with no significant differences in arterial vasorelaxation between the three groups achieving a maximal relaxation of 85±3% control, 82±2% chocolate-supplemented, and 84±3% fenofibrate-treated groups, respectively (Fig. 4B).

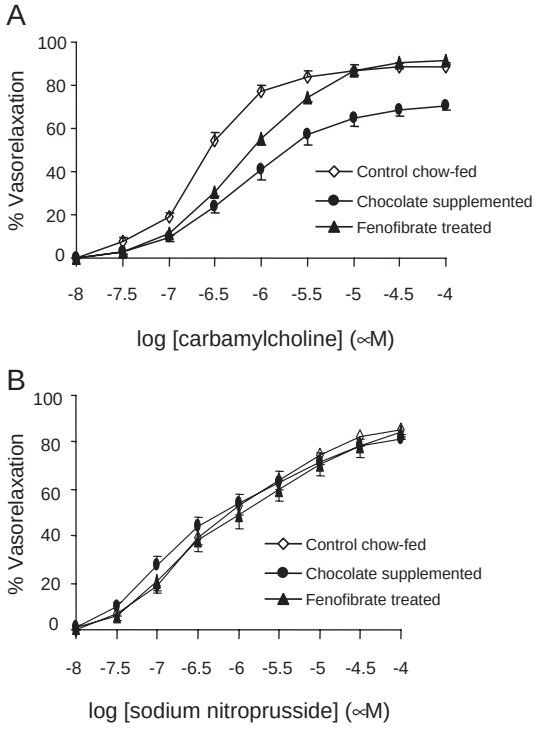

Fig. 4. The effects of (A) carbamylcholine, and (B) sodium nitroprusside on NA-precontracted (8 μM) arteries from three groups of rats. When contraction reached a plateau after 2 min, concentration–response curves to carbamylcholine or sodium nitroprusside were carried out. Data represent mean±S.E.M. The concentration–response curves to carbamylcholine but not to sodium nitroprusside, were significantly reduced in chocolate-supplemented group (ANOVA, $P<0.01$), while fenofibrate treatment significantly ($P<0.01$) improved carbamylcholine-induced vasorelaxation in chocolate-supplemented animals.

4. Discussion

Excessive availability of high-energy diet in many industrialised societies, is producing a health hazard, namely obesity and obesity related secondary disorders, which ultimately leads to the deterioration of quality of life, and in many cases to premature and sudden death. Numerous experimental studies have reported severe detrimental outcome of excessive high-energy diets on well being. For example, high-fat, high-energy diet impairs endothelium-dependent vasorelaxation (Steinberg et al., 1996; Vogel et al., 1997; Naderali and Williams, 2001,2003), while removal of high-energy diet provides some protection against obesity-induced metabolic and cardiovascular abnormalities (Verhamme et al., 2002; Naderali et al., 2004; Sasaki et al., 2002).

In hypertensive obese patients, acetylcholine-induced forearm blood flow is significantly lower than healthy individuals. Caloric restriction decreases body weight and the mean blood pressure, fasting plasma insulin, total cholesterol, triglycerides, low-density lipoproteins as well as enhancing acetylcholine-induced forearm blood flow (Sasaki et al., 2002). Moreover, animal studies have shown that withdrawal of high-energy diet results in significant reduction of plasma lipid profile and a marked improvement of vascular reactivity to acetylcholine (Verhamme et al., 2002; Naderali et al., 2004). Furthermore, pharmacological lowering of circulating plasma lipids has also been shown to reduce the risk for coronary events, cardiovascular morbidity and mortality in a broad range of patients (Brown et al., 1993; Bucher et al., 1999).

In this study, supplemental feeding of animals with chocolate did not result in obesity (characterised with an excessive increase in total body weight and fat pad mass). This may be due to comparable caloric intake between chow-fed control and chocolate-supplemented groups. However, there were significant metabolic abnormalities, namely higher plasma lipids of triacylglycerol and non-esterified fatty acids, in chocolate-supplemented animals. In agreement

with previous reports (Despres et al., 2002; Capell et al., 2003), fenofibrate treatment in the presence of chocolate diet completely reversed lipid profile. Higher plasma lipids seen in this study were similar to those achieved by chronic consumption of high-fat, high-energy diets and obesity (Naderali et al., 2001, 2004; Perticone et al., 2001; Steinberg et al., 1996; Verhamme et al., 2002) indicating that adipose tissue accumulation is not a pre-requisite for abnormal lipid profile (Vogel et al., 1997; Naderali and Williams, 2003).

Examination of contractile and relaxation properties of arteries showed striking differences in responses to various stimuli between the three groups. In contrast to previous reports (Walker et al., 1997; Fontes et al., 1998; Naderali et al., 2001, 2004), contractile responses to KCl and noradrenaline were significantly augmented in chocolate-supplemented diet. Although from our study it is not possible to contemplate the mechanism of hyperreactivity to KCl and noradrenaline, nonetheless, one possibility is that long-term consumption of chocolate may have a direct effect on the activity of the contractile apparatus, or it may have increased/eased the availability of the calcium for contraction required by KCl and noradrenaline. The latter theory is more plausible as chocolate has been shown to markedly increase calciuria (Nguyen et al., 1994). However, these hypotheses merit further investigation.

Numerous studies have shown that excessive high-energy diet impairs endothelium-dependent arterial function (Sasaki et al., 2002; Brown et al., 1993; Bucher et al., 1999; Naderali et al., 2001, 2004; Walker et al., 1997; Fontes et al., 1998). Moreover, defects in vasorelaxation can also be seen with short-term consumption of high-energy diet in the absence of any obesity (Vogel et al., 1997; Naderali and Williams, 2001, 2003). In agreement with previous reports, in this study there was a marked attenuation of endothelial mediated carbamylcholine-induced vasorelaxation in the absence of any obesity, suggesting a significant defect in either carbamylcholine-induced NO production via eNOS activity (Boulanger, 1999) or the release of endothelium-derived hyperpolarizing factor (Gerber et al., 1998). On the other hand, vasorelaxant effect of sodium nitroprusside is due to its direct stimulatory action on cGMP and thereby induction of NO generation (Azula et al., 1996) in vascular smooth muscle cells. Failure of the chocolate-supplemented diet in altering sodium nitroprusside-induced vasorelaxation, argues against any detrimental changes in arterial smooth muscle activity.

A number of various hypotheses have been put forward as the mechanism(s) of action of high-fat diet and obesity related vascular dysfunction, which include a rise in plasma non-esterified fatty acids (Steinberg et al., 1997) hypertriglycerideamia (Tka et al., 1997; Lewis et al., 1999; Naderali et al., 2001). The detrimental effects of increased circulating plasma lipids has been, at least partly, attributed to inhibition of eNOS activity and thereby reduction in NO synthesis (Liao et al., 1995), ultimately resulting in a reduced endothelial-dependent vasorelaxation. Raised non-esterified fatty acids and triacylglycerols levels in chocolate-supplemented animals seen in this study, argue for the role of plasma non-esterified fatty acids and triacylglycerols as the possible agents effecting vasorelaxation. These hypotheses are further strengthened by the finding that fenofibrate treatment in our study improved carbamylcholine-induced responses. Although the mechanism of action of fenofibrate on vascular function is not yet fully known, the protective effects of fenofibrate on vascular function may be due to correction of lipid profile (Despres et al., 2002; Capell et al., 2003) or increasing formation, availability and duration of NO action (Haak et al., 1998; Playford et al., 2002), indicating improvement of endothelial function in fenofibrate-treated animals. Overall, this study confirms that consumption of a high-fat diet attenuates arterial relaxation in the absence of any obesity in the rat. The arterial dysfunction may be due to an induction of hyperlipidaemia in general or hypertriglyceridaemia in particular. Moreover, lowering circulating plasma non-esterified fatty acids and/or triacylglycerols by fenofibrate treatment may have beneficial effects on endothelial function.

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