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Dietary substitution of SFA with MUFA within high-fat diets attenuates hyperinsulinaemia and pancreatic islet dysfunction

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Abstract

Preliminary evidence has suggested that high-fat diets (HFD) enriched with SFA, but not MUFA, promote hyperinsulinaemia and pancreatic hypertrophy with insulin resistance. The objective of this study was to determine whether the substitution of dietary MUFA within a HFD could attenuate the progression of pancreatic islet dysfunction seen with prolonged SFA-HFD. For 32 weeks, C57BL/6J mice were fed either: (1) low-fat diet, (2) SFA-HFD or (3) SFA-HFD for 16 weeks, then switched to MUFA-HFD for 16 weeks (SFA-to-MUFA-HFD). Fasting insulin was assessed throughout the study; islets were isolated following the intervention. Substituting SFA with MUFA-HFD prevented the progression of hyperinsulinaemia observed in SFA-HFD mice ($P < 0{\cdot}001$). Glucose-stimulated insulin secretion from isolated islets was reduced by SFA-HFD, yet not fully affected by SFA-to-MUFA-HFD. Markers of β-cell identity (*Ins2*, *Nkx6.1*, *Ngn3*, *Rfx6*, *Pdx1* and *Pax6*) were reduced, and islet inflammation was increased (IL-1β, 3·0-fold, $P = 0{\cdot}007$; CD68, 2·9-fold, $P = 0{\cdot}001$; *Il-6*, 1·1-fold, $P = 0{\cdot}437$) in SFA-HFD – effects not seen with SFA-to-MUFA-HFD. Switching to MUFA-HFD can partly attenuate the progression of SFA-HFD-induced hyperinsulinaemia, pancreatic inflammation and impairments in β-cell function. While further work is required from a mechanistic perspective, dietary fat may mediate its effect in an IL-1β–AMP-activated protein kinase α1-dependent fashion. Future work should assess the potential translation of the modulation of metabolic inflammation in man.

Key words: **Diet-induced obesity: Insulin secretion: Islets: β-Cell identity: Metabolic inflammation**

Hyperinsulinaemia is a highly pathogenic physiological state, characteristic of obesity and insulin resistance, preceding the onset of pancreatic islet dysfunction and overt type 2 diabetes[1]. High-fat diets (HFD) promote obesity, hyperinsulinaemia and metabolic inflammation[2]. However the composition of fatty acids within HFD alters the impact of dietary fat on insulin biology[3,4]. Feeding SFA-enriched HFD (SFA-HFD) primed and activated nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3 (NLRP3)-mediated IL-1β activation and insulin resistance in adipose tissue, concomitant with hyperinsulinaemia and pancreatic hypertrophy compared with MUFA-enriched HFD[5]. Although previous work from our team suggested that switching from SFA to MUFA-HFD (SFA-to-MUFA-HFD) attenuated the increment in fasting insulin levels[5], that study was not ideal in that a healthy, low-fat-diet (LFD) control group phenotype was not within the study design. Other studies have reported that replacing dietary SFA (palmitate) with high MUFA (oleate) intake reduced inflammatory cytokine secretion[6] and improved insulin sensitivity in women[7]. Importantly, understanding of putative, inflammatory-related mechanisms underpinning the differential effects of SFA-HFD *v.* MUFA-HFD on pancreatic islet function has remained elusive[5].

Inflammation in metabolic tissues plays a critical role in peripheral insulin resistance and is influenced by dietary constituents, including fatty acids[2,3]. In obesity, proinflammatory cytokines, including IL-1β and IL-6, disrupt normal cellular signalling and metabolic pathways[2,8]. Pancreatic inflammation

Abbreviations: AMPK, AMP-activated protein kinase α1; HFD, high-fat diet; LFD, low-fat diet; NLRP3, nucleotide-binding domain, leucine-rich-containing family, pyrin domain-containing-3.

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reduces islet insulin secretion, and chronically elevated IL-1β production/secretion promotes β-cell apoptosis and dysfunction[9–11]. Masters *et al.*[12] also demonstrated that amylin (the main constituent of pancreatic amyloid deposits in type 2 diabetes) activated NLRP3-mediated IL-1β production. Moreover, palmitate is a potent SFA, which is well known to prime and activate IL-1β in an NLRP3-dependent fashion[13,14]. Interesting *in vitro* β-cell studies have shown that MUFA exposure can prevent SFA-induced apoptosis and impairments in cell proliferation[15]. These varying effects of SFA *v.* MUFA warrant further investigation.

The present study has addressed the hypothesis that substitution of dietary MUFA attenuates the adverse effects of SFA-HFD on pancreatic islet function and differentiation. We used a regression feeding model where mice switched from SFA-HFD to MUFA-HFD (i.e. SFA-to-MUFA-HFD) were compared with mice maintained on an SFA-HFD and to healthy LFD control mice, an important extension from previous work in the field. We show that SFA-HFD reduced markers of pancreatic β-cell identity (e.g. *Ins2*, *Nkx6.1*, *Ngn3*, *Rfx6*, *Pdx1* and *Pax6*)[16], coincident with increased inflammation and *in vivo* hyperinsulinaemia; these effects were either partially or fully attenuated in SFA-to-MUFA-HFD mice. This study provides important evidence that dietary MUFA can offset the detrimental effects of prolonged SFA-HFD on pancreatic function and inflammation. Our findings also further highlight the importance of examining the type of dietary fat composition, rather than quantity alone, when considering overall metabolic health.

Materials and methods

Materials and cell culture reagents

Cell culture solutions were purchased from Lonza. All other reagents, unless otherwise stated, were purchased from Sigma-Aldrich.

Animals

Male C57BL/6J mice (aged 7–9 weeks) were purchased from Harlan UK Ltd. Ethical approval was obtained from the University College Dublin Ethics Committee (P15-35), and mice were maintained according to the regulations of the Health Products Regulatory Authority (Directive 2010/63/EU and Irish Statutory Instrument 543 of 2012). Mice were randomly assigned to treatment groups and fed one of three study diets: (1) LFD (10 % energy; *n* 10) for 32 weeks; (2) SFA-based HFD for 32 weeks (SFA-HFD; 45 % energy; *n* 10); or (3) SFA-based diet for 16 weeks followed by a MUFA-based diet for an additional 16 weeks (SFA-to-MUFA-HFD; 45 % energy; *n* 10). The experimental model is depicted in online Supplementary Fig. S1. All study diets were purchased from Research Diets Inc. (catalogue nos. D12450B, D07081501, D07062503, respectively) and represent a reasonable amount of dietary fat expected in the human population. Diet composition and fatty acid profiles are presented in online Supplementary Tables S1 and S2. Body weight and food intake were monitored weekly. Upon completion of the study, mice were euthanised by cervical dislocation.

Metabolic phenotyping of mice

Insulin secretory response was assessed in overnight-fasted mice, where tail blood samples were collected at indicated time points after injection of glucose (25 % w/v, 1·5 g/kg intraperitoneally (i.p.); B. Braun Medical). Insulin concentration was measured by ELISA (Crystal Chem). For glucose and insulin tolerance tests, mice were fasted for 6 h prior to the injection of glucose (25 % w/v, 1·5 g/kg i.p.) or insulin (0·5 U/kg; Actrapid, Novo Nordisk), respectively. Glucose levels were monitored at indicated time points using a blood glucometer from Accu-Check (Roche). Throughout the study, fasting insulin and glucose levels were also measured by ELISA or glucometer, respectively, at weeks 0, 16, 20, 24 and 32.

Pancreatic immunostaining and isolation of islets

Following euthanasia, either pancreatic immunostaining or islet isolation was conducted. For immunostaining, mouse pancreata were removed and fixed in 10 % neutral balanced formalin prior to dehydration and paraffin-embedding. Tissues were sectioned to obtain 8-μm slices using a microtome (Leica RM 2135). Sections were rehydrated, followed by antigen retrieval and the addition of a hydrophobic barrier using a dako pen. Samples were blocked with 10 % bovine serum albumin, washed and incubated in the dark at 4°C with primary antibodies IL-1β (Abcam Ab9722; 1 μg/ml) and CD68 (Abcam Ab53444; 2 μg/ml). The next day, fluorescent secondary antibodies were added for 1 h, with the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI) included during the final 20 min of incubation. Sections were imaged using a Zeiss Axio Imager M1 and quantified using AxioVision (Zeiss) and ImageJ software.

For islet isolation, the pancreas was perfused through the pancreatic duct with collagenase (SERVA) and the tissue digested as previously described[17]. Islet separation was completed using two Histopaque density gradients (Sigma-Aldrich), and islets were hand-picked and cultured in Roswell Park Memorial Institute (RPMI) medium supplemented with 10 % heat-inactivated fetal bovine serum and 1 % penicillin-streptomycin[17,18].

Assessment of islet function

Glucose-stimulated insulin secretion from isolated islets was assessed as described previously[18–20]. Briefly, after culturing islets in Krebs–Ringer–HEPES–bicarbonate solution (10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid), 140 mM NaCl, 3·6 mM KCl, 2 mM $NaHCO_3$, 0·5 mM NaH_2PO_4, 0·5 mM $MgSO_4$, 1·5 mM $CaCl_2$, and 0·1 % bovine serum albumin, pre-equilibrated with 95 %:5 % O_2:CO_2 and pH 7·4) with 6 mM glucose for 1 h at 37°C, batches of ten islets per mouse were then transferred to Krebs–Ringer–bicarbonate–HEPES supplemented with either low (6 mM) or high (17 mM) glucose for 30 min at 37°C[19,20]. Secreted and total insulin fractions were analysed by ELISA. The insulin secretion stimulatory index was obtained as a ratio of insulin secreted under high/low glucose conditions.

Real-time RT-PCR

Whole liver tissue was collected after sacrifice, snap-frozen in liquid nitrogen and stored at –80°C prior to tissue

homogenisation. Total RNA from islets or liver was extracted using TriReagent (Sigma-Aldrich) as per manufacturer's instructions, and quantified using a Nanodrop (ThermoFisher Scientific)[5]. Single-stranded complementary DNA was synthesised from 1 μg of total RNA using a high-capacity complementary DNA Reverse Transcription Kit (ThermoFisher Scientific), and real-time RT-PCR was conducted using a QuantStudio 7 Flex RT-PCR system and Taqman Gene Expression Master Mix with SYBR Green (ThermoFisher ABI). Primer details are provided in online Supplementary Table S3. Changes in gene expression were determined using the ΔΔCt normalisation and quantification method[21], where *β*-actin (*Actb*) and 18s were used as housekeeping genes for islets and liver, respectively. Both reference genes were highly stable in their respective tissues (<2·4 and <2·7 % variability). All primers were purchased from Applied Biosystems (ThermoFisher Scientific).

Statistical analyses

Data are reported as mean values with their standard errors. Sample size for detecting a significant difference between groups (insulin concentration as main parameter) was calculated using G*Power (3.1.9.2) assuming an effect size of 1·27, a type I error of 0·05 (two tails) and statistical power of 0·80. To analyse metabolic phenotype data with multiple time points (insulin secretion response, glucose tolerance test, insulin tolerance test, changes in fasted insulin and homeostasis model assessment of insulin resistance over time[22]), we performed two-way repeated-measures ANOVA to test for differences between groups. When an ANOVA was significant, Bonferroni-corrected *post hoc* comparisons were examined. AUC analysis was performed on curves from the insulin secretion response using GraphPad Prism 5 software. For between-group comparisons at a single time point, one-way ANOVA was performed with Bonferroni-corrected *post hoc* comparisons when an ANOVA was significant. GraphPad Prism 5 was used for all statistical analyses. A P value <0·05 was considered statistically significant, with significant comparisons described in the captions of all figures.

Results

Switching to MUFA-high-fat diet attenuated hyperinsulinaemia regardless of changes in body weight

In order to determine whether MUFA-HFD may offset the impact of SFA-HFD, we used a regression feeding model wherein mice were first fed an SFA-HFD for 16 weeks to induce obesity and hyperinsulinaemia. Half of the SFA-HFD mice were then switched to MUFA-HFD, and this group (SFA-to-MUFA-HFD) was compared with SFA-HFD and age-matched LFD control mice. Interestingly, switching from SFA-to-MUFA-HFD after 16-week HFD prevented further elevations in fasting insulin levels over time as was observed in mice maintained on SFA-HFD ($P < 0·001$; n 15–30; Fig. 1(a)). Nonetheless, the SFA-to-MUFA-HFD group remained hyperinsulinaemic compared with the LFD group, indicating that MUFA intervention could block further progression of disease but did not completely regress the adverse phenotype. Differences in circulating insulin between HFD groups remained significant after weight-matching mice (data not shown). Furthermore, switching from SFA-to-MUFA-HFD also improved homeostasis model assessment of insulin resistance and homeostasis model assessment of insulin sensitivity over time (Fig. 1(a) and (c)). There were no significant differences in homeostasis model assessment-*β*-cell function between SFA-HFD and SFA-to-MUFA-HFD groups (data not shown).

Interestingly, upon completion of the study (week 32), the insulin secretion response to glucose injection was attenuated in the SFA-to-MUFA-HFD group compared with SFA-HFD ($P < 0·001$; n 15–30; Fig. 1(d)). Both HFD groups had an elevated insulin secretion response compared with LFD. Similarly, the insulin secretion response AUC was significantly reduced in SFA-to-MUFA-HFD mice, compared with feeding the SFA-HFD alone ($P < 0·0001$; Fig. 1(e)). There was no difference in the insulin secretion response or corresponding AUC at week 16, prior to the dietary switch (data not shown). In terms of adjusting for differences in fasting insulin concentrations, the incremental insulin secretion AUC in SFA-to-MUFA-HFD mice was similar to the LFD, with both groups being significantly lower than SFA-HFD mice ($P = 0·0013$ and 0·0007, respectively; n 10–11; Fig. 1(f)). Also, in terms of weight gain, at week 16 prior to the dietary switch, there were no significant differences in body weight between mice assigned to SFA-to-MUFA-HFD or maintained on SFA-HFD, nor were there differences in caloric intake (online Supplementary Fig. 2(e) and (f)). However, by the end of the intervention (week 32), SFA-HFD mice had gained more weight than SFA-to-MUFA-HFD mice ($P = 0·0008$; n 10; Fig. 1(g)). Nevertheless, despite weight matching, the insulin secretion response remained more profound in SFA-HFD mice compared with the SFA-to-MUFA-HFD group (Fig. 1(h)). Glucose and insulin tolerance tests were not different between SFA-HFD and SFA-to-MUFA-HFD groups (online Supplementary Fig. S2(a) and (b)), indicating no difference in insulin resistance between HFD groups.

Switching from SFA to MUFA-high-fat diet attenuated the adverse effects of SFA-high-fat diet on markers of β-cell function, metabolism and differentiation

To examine whether the dietary fat composition induced changes in islet function, we assessed glucose-stimulated insulin secretion from isolated islets. Interestingly, the insulin stimulatory index was markedly reduced in islets isolated from SFA-HFD mice, compared with LFD mice ($P = 0·002$; n 10; Fig. 2(a)). However, despite the onset of obesity in both HFD groups, the reduction in insulin stimulatory index was not as profound in islets from SFA-to-MUFA-HFD mice ($P = 0·078$; n 10; Fig. 2(a)). There were no significant differences in basal islet insulin secretion between diet groups (online Supplementary Fig. S2(c)).

In terms of understanding the molecular perturbations induced in pancreatic islets, *Ins2* mRNA expression was significantly reduced after SFA-HFD ($P = 0·003$ *v.* LFD; n 10; Fig. 2(b)), but this was preserved by switching mice from SFA-to-MUFA-HFD. Similarly, islet *Ampk* (*Prkaa1*) mRNA expression was markedly reduced in SFA-HFD mice, compared with the LFD group ($P = 0·004$), an effect that was partially prevented by

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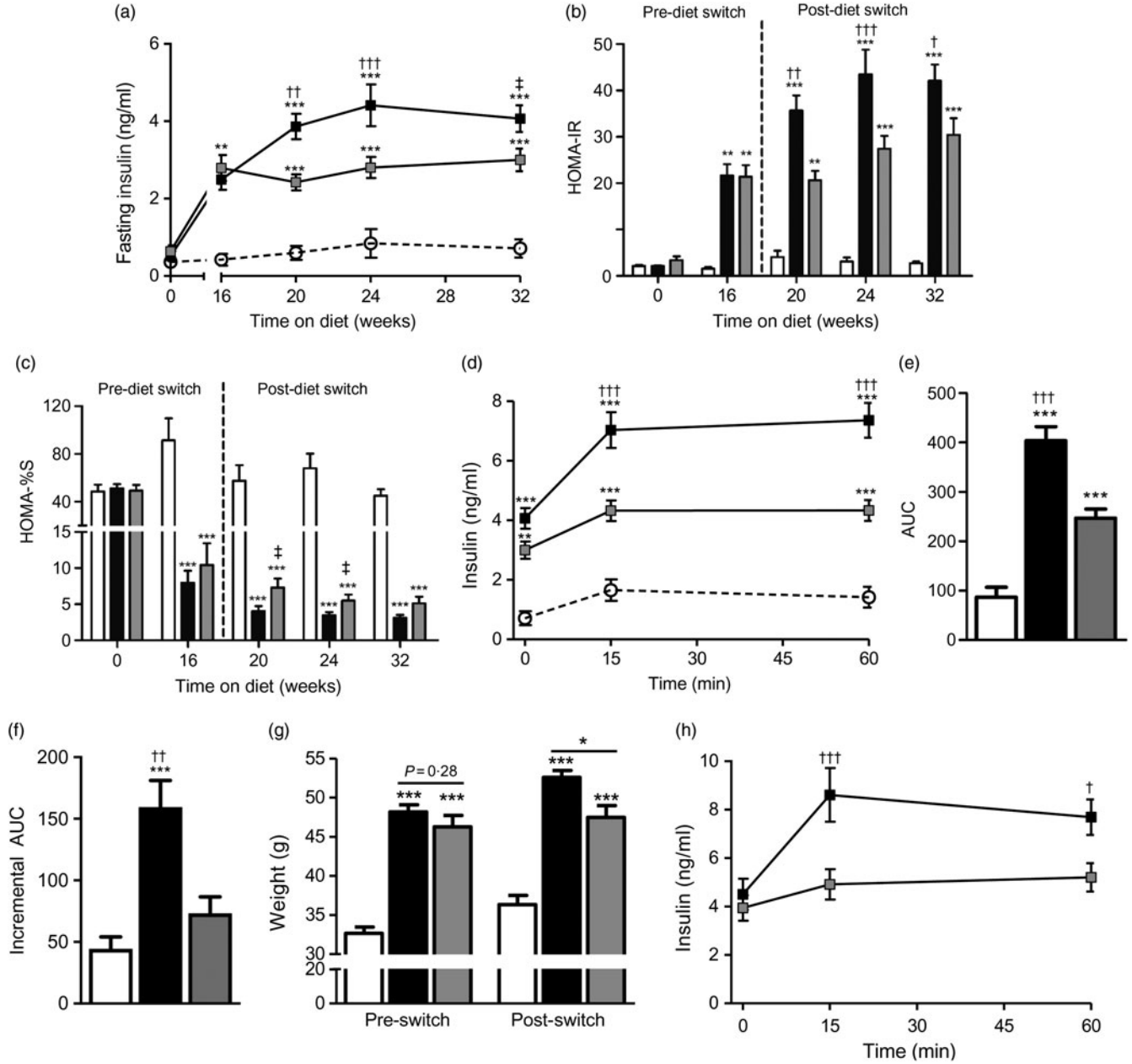

Fig. 1. Effects of a dietary switch from SFA-to-MUFA-high-fat diet (HFD) on metabolic phenotype. Fasting insulin levels (a), homeostatic model assessment of insulin resistance (HOMA-IR) (b) and homeostatic model assessment of insulin sensitivity (HOMA-%S) (c) were tracked throughout the dietary intervention. Insulin secretion response was examined in overnight-fasted mice (*n* 15–30) with intraperitoneal injection of 1·5 g/kg glucose (d), and corresponding AUC (e) and incremental AUC (f) are shown. Mice weights are indicated in (g). Given a difference in body weight at week 32, the insulin secretion response was also assessed in weight-matched groups (h). (a–h), $^{*}P<0{\cdot}05$, $^{**}P<0{\cdot}01$, $^{***}P<0{\cdot}001$ *v.* low-fat diet (LFD); †$P<0{\cdot}05$, ††$P<0{\cdot}01$, †††$P<0{\cdot}001$, SFA-HFD *v.* SFA-to-MUFA-HFD; by two-way (a–d, h) or one-way (e–g) ANOVA with Bonferroni *post hoc* comparisons; ‡ $P<0{\cdot}05$ SFA-HFD *v.* SFA-to-MUFA-HFD by unpaired Student's *t* test. (a, d, h) –⊖-, LFD; –■–, SFA-HFD; –□–, SFA-to-MUFA-HFD; (b, c, e, f, g) □, LFD; ■, SFA-HFD; ▭, SFA-to-MUFA-HFD.

switching from SFA-to-MUFA-HFD ($P=0{\cdot}029$; *n* 10; Fig. 2(b)). However, despite these differences, the expression of *Ldha* (a *β*-cell 'disallowed' or selectively repressed gene sensitive to *Ampk*[23–25]) was not significantly changed (Fig. 2(b)).

We next assessed whether switching from SFA-to-MUFA-HFD could alter the expression of *β*-cell-enriched transcription factors and key genes that maintain *β*-cell identity. The expressions of *Nkx6.1*, *Ngn3* and *Rfx6* were all significantly reduced in mice in the SFA-HFD group compared with LFD ($P=0{\cdot}043$, 0·042 and 0·046, respectively; *n* 10; Fig. 2(c)). Conversely, the expression of this panel of genes was significantly elevated in SFA-to-MUFA-HFD mice compared with SFA-HFD mice, to levels that were not significantly different from LFD mice ($P=0{\cdot}017$, 0·007 and 0·011 *v.* SFA-HFD for *Nkx6.1*, *Ngn3* and *Rfx6*, respectively; *n* 10; Fig. 2(c)). *Pdx1* and *Pax6* mRNA levels showed a similar response, albeit not statistically significant. Dietary modifications did not alter islet *MafA* expression.

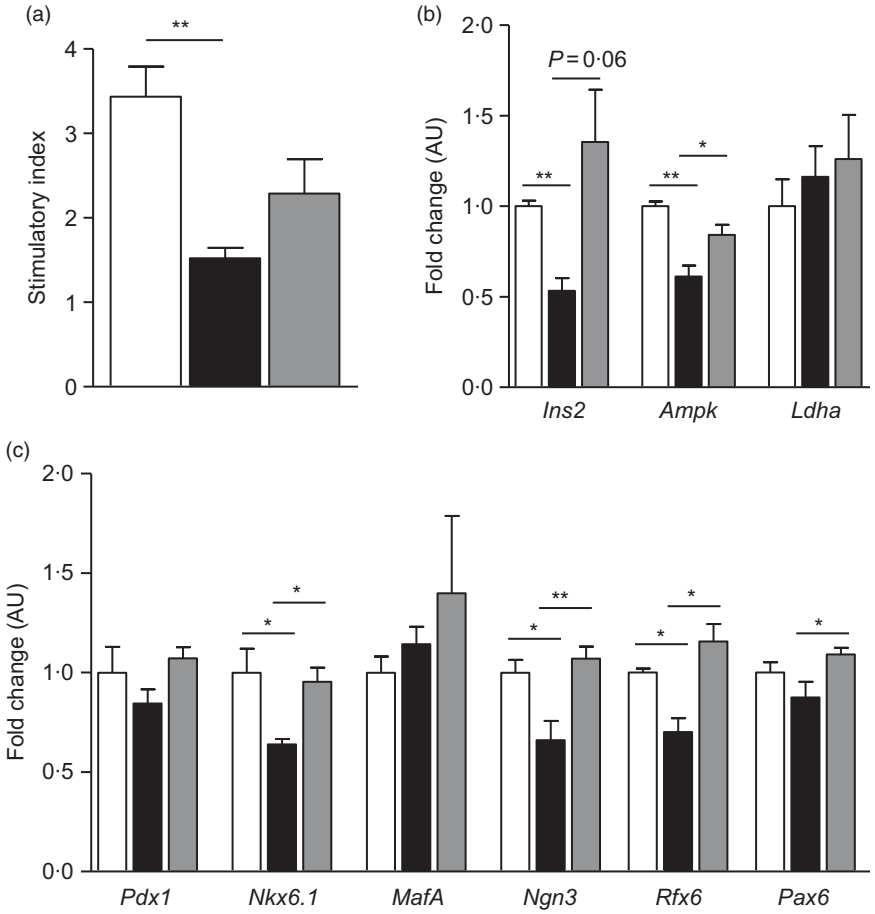

Fig. 2. Effects of a dietary switch from SFA-to-MUFA-high-fat diet (HFD) on pancreatic islet function. Insulin stimulatory index (a), and markers of β-cell identity and differentiation (b and c) were examined. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ between indicated comparisons by one-way ANOVA with Bonferroni *post hoc* comparisons. AU, arbitrary units; *Ins2*, insulin II; *Ampk*, AMP-activated protein kinase α1; *Ldha*, lactate dehydrogenase A; *Pdx1*, pancreatic and duodenal homeobox 1; *Nkx6.1*, NK6 homeobox 1; *MafA*, pancreatic β-cell-specific transcriptional activator; *Ngn3*, neurogenin 3; *Rfx6*, regulatory factor X6; *Pax6*, paired box gene 6. (a–c) ☐, low-fat diet; ■, SFA-HFD; ▭, SFA-to-MUFA-HFD.

The liver plays a key role in insulin clearance and degradation. Consequently, we assessed hepatic *Ceacam1* expression, as the deletion of this gene causes hyperinsulinaemia due to impaired insulin clearance in addition to increased lipogenic gene expression and insulin resistance[26,27]. Hepatic *Ceacam1* was most reduced by SFA-HFD, an effect that was not fully affected by switching to MUFA-HFD (Fig. 3). Similarly, hepatic *Irs-2* mRNA was lowered by both HFD irrespective of fatty acid composition (Fig. 3). Other lipogenic genes, including *Acc-α*, *Fasn* and *Scd1*, were not markedly altered between diets (online Supplementary Fig. S2(d)).

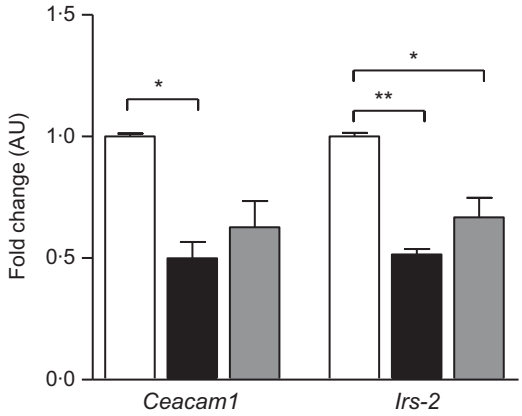

Fig. 3. Hepatic gene expression of *Ceacam1* and *Irs-2*. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ between indicated comparisons by one-way ANOVA with Bonferroni *post hoc* comparisons. AU, arbitrary units; *Ceacam1*, carcinoembryonic antigen-related cell adhesion molecule 1; *Irs-2*, insulin receptor substrate 2. ☐, Low-fat diet; ■, SFA-high-fat diet (HFD); ▭, SFA-to-MUFA-HFD.

Pancreatic inflammatory markers were significantly reduced in mice switched from SFA-to-MUFA-high-fat diet

Palmitate is a potent trigger of IL-1β signalling, which can promote pancreatic inflammation and β-cell dysfunction[28]. Immunostaining results demonstrated enhanced expression of both IL-1β ($P = 0{\cdot}007$; Fig. 4(a) and (b)) and the macrophage marker CD68 ($P = 0{\cdot}001$; Fig. 4(c) and (d)) in the SFA-HFD group

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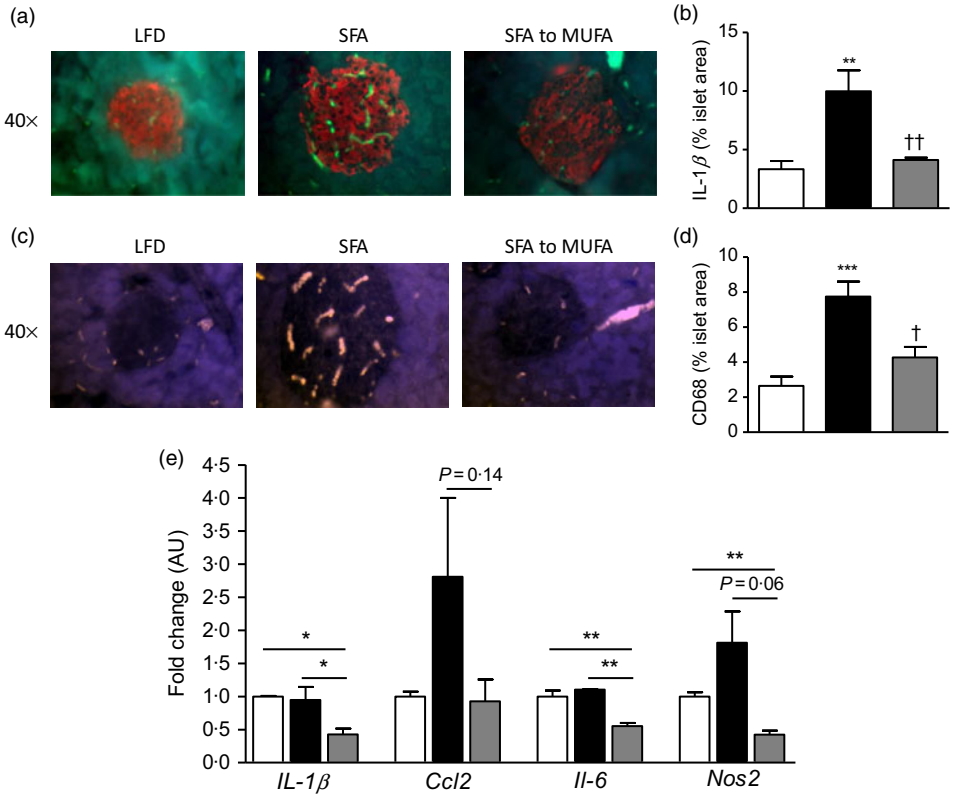

Fig. 4. Effects of a dietary switch from SFA-to-MUFA-high-fat diet (HFD) on pancreatic inflammatory markers. Immunostaining results for IL-1β (green) (a and b) and the macrophage marker CD68 (orange) (c and d). Expressions of inflammatory markers are shown in (e) (n 10). (b and d), $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$ *v.* low-fat diet (LFD); $\dagger P < 0{\cdot}05$, $\dagger\dagger P < 0{\cdot}01$, SFA-HFD *v.* SFA-to-MUFA-HFD; (e), $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, between indicated comparisons by one-way ANOVA with Bonferroni *post hoc* comparisons. CD68, cluster of differentiation 68; AU, arbitrary units; *Ccl2*, monocyte chemoattractant protein 2; *Nos2*, nitric oxide synthase 2. (b, d, e) ☐, LFD; ■, SFA-HFD; ▭, SFA-to-MUFA-HFD.

compared with the LFD group. Remarkably, elevated IL-1β and CD68 expression was attenuated when mice were switched from SFA-to-MUFA-HFD ($P = 0{\cdot}008$ and 0·011, respectively; Fig. 4(a)–(d)).

To validate the immunostaining results, we also determined islet gene expression of *Il-1β*, *Il-6* and *Nos2*. Islet gene expression of *Il-1β*, *Il-6* and *Nos2* was consistently lower in SFA-to-MUFA-HFD mice compared with age-matched LFD and SFA-HFD groups ($P = 0{\cdot}011$ *v.* LFD, 0·034 *v.* SFA; 0·005 *v.* LFD; and $P = 0{\cdot}001$ *v.* SFA; 0·003 *v.* LFD, 0·057 *v.* SFA, respectively; n 10; Fig. 4(e)).

Discussion

Our study demonstrated that, in comparison with continuous consumption of SFA-HFD, switching to MUFA-HFD partially preserved the expression of markers of β-cell identity and differentiation, coincident with reduced pancreatic inflammation and attenuated impairments in islet function. We observed a very consistent pattern of changes in islet gene expression, wherein SFA-HFD significantly reduced markers of β-cell differentiation, proliferation and identity (e.g. *Ins2*, *Nkx6.1*, *Ngn3* and *Rfx6*, and trends for *Pdx1* and *Pax6*), the down-regulation of which has been associated with impaired cell function[16,29]. Conversely, yet just as consistently, the same β-cell markers were not adversely affected after switching to MUFA-HFD and were similar to the LFD group. These findings extend previous *in vitro* work showing that MUFA exposure in human pancreatic β-cells prevented SFA-induced apoptosis and impairments in β-cell proliferation[15]. It appears that SFA-HFD weakens β-cell differentiation, whereas replacement of SFA with dietary MUFA prevents these detrimental effects and maintains differentiation potential at levels seen in healthy LFD-fed mice. Furthermore, the aforementioned effects likely contributed to the coinciding differences in islet insulin secretory capacity.

While our data suggest that fat quality may affect islet functionality, we need to acknowledge a potential impact of body weight. There was a small but significant difference in weight between SFA-HFD and SFA-to-MUFA-HFD groups at week 32. Nevertheless, when we weight-matched the insulin secretion response, there was a clear difference between groups based on fatty acid composition. In terms of potential differences between fatty acids, SFA, specifically palmitate, promotes inflammation in adipose tissue[5] and pancreatic islets[15]. However, the impacts of different fatty acids are much less defined in the pancreas compared with adipose tissue[2]. We therefore assessed pancreatic inflammation with a view to understand the mechanisms driving SFA-HFD-induced impairments in islet gene expression. In this study, SFA-HFD significantly increased both IL-1β and the macrophage marker CD68 in islets, yet this effect was prevented in the SFA-to-MUFA-HFD group.

Furthermore, changes in inflammatory gene expression mirrored immunostaining results, where MUFA-HFD reduced *Il-6*, *Nos2* and *Il-1β*. Recent work by Nordmann *et al.*[28] demonstrated in isolated islets that another common SFA, stearate, acted similarly to IL-1β and IL-6 to significantly reduce markers of β-cell differentiation, including *Pdx1* and *Nkx6.1*. Moreover, anti-inflammatory treatments, including anti-IL-1β antibody, anti-TNFα antibody and sodium salicylate, improved isolated islet insulin secretion[28].

Our *ex vivo* islet work extends and corroborates this concept of attenuating inflammation by dietary manipulation to protect islet biology. We demonstrated that a significant reduction in the insulin secretion stimulatory index from isolated islets of mice fed SFA-HFD was attenuated in mice switched from SFA-to-MUFA-HFD. This concurs with the work of Maedler *et al.*[15], which showed that *ex vivo* glucose-stimulated insulin secretion from human islets was completely abolished upon exposure to palmitate (0·5 mM for 4 d), whereas glucose-stimulated insulin secretion was completely restored by the addition of MUFA. Furthermore, Gerst *et al.*[30] suggested that diabetogenic factors, including palmitate, target both pancreatic β-cells as well as pancreatic pre-adipocytes and adipocytes to promote inflammation, and this combination may accelerate β-cell failure. Taking these data together, we speculate that pancreatic inflammation observed in SFA-HFD-fed mice was attributable to the proinflammatory effects of pancreatic adipocytes and/or SFA-induced NLRP3-mediated IL-1β destruction of islet function[12,30], which did not occur with the less inflammatory MUFA-enriched HFD.

Counterintuitively, we observed the presence of hyperinsulinaemia and enhanced *in vivo* insulin secretion response in SFA-HFD-fed mice despite β-cell dedifferentiation and reduced *ex vivo* islet insulin secretion; however, this may be explained by coincident inflammation. Indeed, genetic or diet-induced models of obesity contribute to both hyperinsulinaemia and pancreatic inflammation[31–36]. Through various mechanisms, including increased fibrosis[32] or elevated islet blood perfusion[32,37], inflammation in the pancreas can contribute to hyperinsulinaemia and downstream tissue dysfunction. Moreover, the elevated pancreatic IL-1β in SFA-HFD mice is fascinating in view of recent work suggesting that, although IL-1β is traditionally detrimental to islet function, it may also promote insulin secretion, highlighting the complexities of IL-1β functionality. Furthermore, IL-1β and systemic insulin appear to promote the secretion of one another[38]. Taken together, it's possible that despite dysfunctional insulin secretion in isolated islets, integrative *in vivo* biology maintains hyperinsulinaemia in SFA-HFD mice, emphasising the importance of using both *ex vivo* and *in vivo* models in animal interventions.

While the pancreas plays a pivotal role in systemic insulin homeostasis, the liver also regulates insulin clearance. The deletion of carcinoembryonic antigen-related cell adhesion molecule 1 (*Ceacam1*) ($Ccl^{-/-}$) causes hyperinsulinaemia due to impaired insulin clearance[26,27]. Typically, upon pancreatic insulin secretion, CEACAM1 associates with the insulin receptor and promotes hepatic insulin clearance and degradation[39]. Interestingly, SFA-HFD-fed mice had significantly reduced hepatic *Ceacam1* expression. Lester *et al.*[27] demonstrated that feeding HFD (45 % fat from lard) down-regulated hepatic *Ceacam1* expression with hyperinsulinaemia.

Undoubtedly, this paper has not addressed all the possible mechanisms in relation to the potential protective effects of MUFA *v.* SFA-HFD on pancreatic function and insulin biology. The area of metabolic-inflammation is far more complex than previously anticipated[2]. It is not a simple paradigm, wherein proinflammatory cytokines impede metabolism, but that metabolic reconfiguration determines the nature of the cellular inflammatory profile[40]. For example, AMP-activated protein kinase α1 (AMPK) is a key regulator of NLRP3-mediated IL-1β activation[41]. In this context, *Ampk* attenuation in SFA-HFD islets concurrent with augmented pancreatic IL-1β and CD68 is noteworthy, since pancreatic AMPK may be necessary to maintain normal glucose-sensing and insulin secretion from β-cells[42]. Thus, elevated *Ampk* in conjunction with lower pancreatic IL-1β in SFA-to-MUFA-HFD islets may partially protect against the overstimulation of β-cell insulin secretion and hyperinsulinaemia. Such bidirectional co-regulation of AMPK and IL-1β aligns with our previous work that focused on the adipose tissue, where MUFA-HFD preserved adipose AMPK and attenuated IL-1β activation compared with SFA-HFD[5]. A loss of β-cell AMPK can dysregulate differentiation and cause misexpression of key 'disallowed genes' (genes selectively repressed in β-cells), including *Ldha*[25,42]. Therefore, lower islet *Ampk* expression, concurrent with inflammation, may contribute to blunted β-cell differentiation in SFA-HFD-fed mice.

All studies have limitations; here a time course element would be insightful. Our islet experiments were only conducted at 32 weeks. Other studies following 8- or 14-week HFD feeding[32,33] caused hyperinsulinaemia, islet inflammation and/or dysfunction, but islet insulin secretory function was still intact or elevated compared with controls. Contrary to this, a later time span reflects long-term dietary impact; 32 weeks was significantly longer to allow for the initial SFA-HFD insult before determining the impact of switching to MUFA-HFD. It is perhaps not surprising that islet secretory function became compromised in SFA-HFD islets after 32 weeks. Future work investigating islet function in response to prolonged diet intervention is warranted in a gender-dependent manner. It is also critical to acknowledge that we only investigated a palmitate-enriched SFA-HFD (16:0), whereas a previous work has shown that varying SFA chain lengths can have differential effects on obesogenic co-morbidities[43,44]. Moreover, switching from SFA-to-MUFA-HFD did not resolve insulin resistance or the obese phenotype despite healthier islet function, further emphasising the complex effects of different fatty acid types on metabolic health. Further investigation into the effects of different SFA and MUFA types on pancreatic and whole-body health is warranted. Finally, further work is needed to support our mechanistic insights in order to unravel the precise molecular pathways underlying the differential effects of MUFA *v.* SFA. In particular, the exploration of pancreatic steatosis and/or inflammatory and apoptotic-dependent pathways on β-cell mass and functionality, as well as the relative contribution of dietary fats on $CD68^+$ immune cells *v.* β-cells, is warranted.

British Journal of Nutrition

Conclusion

In summary, this study highlights that switching to MUFA-HFD prevented further progression of SFA-HFD-induced inflammation in pancreatic islets. MUFA-HFD partly attenuated hyperinsulinaemia compared with SFA-HFD, an important consideration since HFD-induced hyperinsulinaemia may further drive obesity-related complications. Collectively, this work highlights that changing the type of dietary fat may have significant implications on pancreatic function and health. While the translational potential needs to be verified in human populations, recent retrospective analyses[4,45] suggest that dietary fat reconfiguration may have a potential to differentially modulate the progression of insulin resistance and diabetes in man.

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Supplementary material

For supplementary materials referred to in this article, please visit https://doi.org/10.1017/S0007114520000859

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