Dietary isoflavone intake is associated with evoked responses to inflammatory cardiometabolic stimuli and improved glucose homeostasis in healthy volunteers

J.F. Ferguson a,*, M.F. Ryan b, E.R. Gibney b, L. Brennan b, H.M. Roche b,c, M.P. Reilly a

a Cardiovascular Institute, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA, USA
b UCD Institute of Food & Health, University College Dublin, Ireland
c Conway Institute, School of Public Health and Population Sciences, University College Dublin, Ireland

Received 18 October 2013; received in revised form 20 March 2014; accepted 24 March 2014
Available online 18 April 2014

KEYWORDS
Cardiometabolic disease; Endotoxemia; LPS; Isoflavone; Soy; Inflammation; Insulin sensitivity

Abstract Background and aims: Consumption of foods that modulate inflammatory stress in genetically-prone individuals may influence development of cardiometabolic diseases. Isoflavones in soy-derived foods function as phytoestrogens, have antioxidant and anti-inflammatory activity, inhibit protein-tyrosine kinase activity, and may be atheroprotective. We examined the relationship between soy food consumption and inflammatory responses to endotoxia, postprandial responses to oral lipid tolerance test (OLTT), and insulin sensitivity from frequently sampled intravenous tolerance tests (FSIGTT).

Methods and results: We administered low-dose endotoxin (LPS 1 ng/kg) to induce transient endotoxia in young, healthy volunteers (N = 215) of African (AA), and European (EA) ancestry as part of the GENE Study. We further supported these findings in two independent samples: the MECHE Study and NHANES. Soy food consumption was a significant predictor of peak cytokine response following LPS. Individuals with moderate-high (>1.48 mg/day, N = 65) vs. low-no (≤1.48 mg/day, N = 150) isoflavone consumption had significantly higher tumor necrosis factor alpha (TNFα) post-LPS (AUC, P = 0.009). Further, high-isoflavone consumers were protected against inflammation-induced decline in insulin sensitivity (SI) in GENE. We observed significant differences by soy consumption in the interferon gamma (IFNγ) response to OLTT, and the insulin response to OGTT in MECHE, as well as significantly lower fasting insulin, and 2-hour glucose post-OGTT in EA NHANES subjects.

Conclusion: We demonstrate that soy consumption may influence inflammatory and metabolic responses. In research of nutritional exposures, measuring evoked phenotypes may be more informative than describing resting characteristics.

The GENE Study was registered under NCT00953667 and the MECHE Study under NCT01172951, both at clinicaltrials.gov.

© 2014 Elsevier B.V. All rights reserved.

Abbreviations: LPS, lipopolysaccharide; FSIGTT, frequently sampled intravenous glucose tolerance test; OGTT, oral glucose tolerance test; OLTT, oral lipid tolerance test; GENE, Genetics of Evoked responses to Niacin and Endotoxemia Study; MECHE, Metabolic Challenge Study; NHANES, National Health and Nutrition Examination Survey; SFA, saturated fatty acids; MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

* Corresponding author. Perelman School of Medicine at the University of Pennsylvania, 11-130-11 Smilow Center for Translational Research, 3400 Civic Center Boulevard, Building 421, Philadelphia, PA 19104, USA. Tel.: +1 215 898 9320.
E-mail address: jfer@mail.med.upenn.edu (J.F. Ferguson).
Introduction

Inflammation is a key component of several cardiometabolic diseases, including obesity, type 2 diabetes (T2D) and atherosclerotic cardiovascular disease (CVD) [1]. While many factors, including genetic, environmental and microbial, influence the development of a pro-inflammatory state, habitual diet may be a key inflammatory regulator [2]. We utilize evoked endotoxemia (LPS) in healthy individuals as a model of physiological responses to inflammatory stimuli, inflammation-induced insulin resistance, and cardiometabolic risk [3–5], with relevance to postprandial metabolic endotoxemia [6]. Dietary components which modulate response to LPS may influence transient postprandial inflammatory stress, affect ability to appropriately resolve inflammatory stimuli, and influence chronic cardiometabolic disease and diet-induced obesity.

Bioactive food compounds may have important health-modulating effects. Dietary plant-derived phytochemicals have anti-inflammatory and antioxidant properties that may be protective against disease development [7]. Isoflavones, primarily genistein (~50–60%), daidzein (~30–40%), and glycitein (~5–10%), are found in high concentrations in soy-derived foods [8]. Isoflavones function as phytoestrogens [9], inhibit protein-tyrosine kinase activity [10], and may have an anti-proliferative effect on cancer cells [11]. Although human epidemiological and interventional data remain inconclusive, mounting evidence suggests that isoflavones may be atheroprotective [12,13].

As part of the Genetics of Evoked Responses to Niacin and Endotoxemia (GENE) Study [4], we administered a low dose of endotoxin (LPS 1 ng/kg) to induce a controlled inflammatory response. We found that dietary isoflavone intake was associated with the inflammatory response to endotoxemia, and with endotoxemia-induced changes in insulin sensitivity. The findings were supported by complementary analyses in two independent samples; the MECHE study (www.ucd.ie/jingo/) and NHANES (www.cdc.gov/nchs/nhanes.htm).

Methods

GENE study population

Details of the GENE Study have been published previously [4]. Briefly, healthy volunteers (N = 294), non-smokers, age 18–45, BMI 18–30 kg/m², African American (AA) or European (EA) ancestry were recruited to an inpatient endotoxin challenge (1 ng/kg LPS), and frequently sampled intravenous glucose tolerance tests (FSIGTT) pre- and post-LPS at the University of Pennsylvania (UPenn) Clinical and Translational Research Center (CTRC). Subjects who completed dietary records (N = 215) were analyzed here. An overview of the GENE-Diet Study is shown in Fig. 1. The GENE study was conducted in accordance with UPenn’s IRB with regulatory oversight by the FDA (LPS: IND# 5984) and an NIH-appointed data-safety and monitoring board.

All subjects provided informed written consent. The GENE Study was registered under NCT00953667 at Clinicaltrials.gov.

GENE dietary analysis

Subjects received counseling from a CTRC dietician at study initiation to ensure adherence to AHA recommendations (55–60% carbohydrate; 10–15% protein; <30% fat; <7–10% SFA; <300 mg cholesterol/day), and were instructed in the use of food records. Subjects completed 3-day records on two separate occasions; before the LPS inpatient visit, and before a separate inpatient visit (±2–4 weeks). Results were averaged across the 6 days of records for each subject to obtain an estimate of habitual consumption, and these averages used in subsequent dietary analysis. Subjects did not receive any specific counseling related to soy food intake and there was no dietary intervention implemented. Subjects who completed all dietary records were included in the current analysis (N = 215 completed). Nutrient data were analyzed using Food Processor 8.1 (ESHA Research, Salem, OR).

GENE LPS inpatient visit

The inpatient LPS visit lasted ~40 h, as described [4]. Following overnight acclimatization, LPS (1 ng/kg) was administered intravenously by a licensed physician investigator. Anthropometric measurements were recorded and multiple clinical variables assessed regularly during the visit. Serial blood draws were taken, and serum and
plasma isolated for measurement of cytokines. Samples were stored at −80 °C prior to analysis.

**GENE frequently sampled intravenous glucose tolerance testing (FSIGTT)**

A 50% glucose bolus (0.3 g/kg) was administered at baseline over a one minute period as described [5]. Insulin (0.03 U/kg) was injected 20 min post-glucose. Blood samples were obtained at 22 time-points throughout the 3-hour FSIGTT, and plasma aliquots stored at −80 °C prior to measurement of insulin and glucose. Data were modeled using MINMOD [14] a computational model which incorporates the insulin and glucose values obtained during FSIGTT to calculate parameters of glucose and insulin sensitivity including the insulin sensitivity index ($S_i$), a measure of the capacity of tissues to take up glucose in response to insulin.

**GENE laboratory analyses:** As described [4] plasma levels of Tumor necrosis factor alpha (TNF-α), Interleukin-6 (IL-6), and Interleukin-1 receptor agonist (IL-1RA) were measured by ELISA (Quantikine, R&D Systems; Minneapolis, MN). Plasma insulin levels were measured in duplicate (radio-immunoassays (RIA), Linco Research, St Charles, MO) according to manufacturers’ guidelines. A Hitachi 912 automated chemistry system was used to measure plasma glucose (Wako Diagnostics, Richmond VA). High-sensitivity C-reactive protein (CRP) was measured on a Behring Nephelometer II Analyzer (Siemens Diagnostics; Munich, Germany).

**Supporting studies**

**MECHE:** The Metabolic Challenge (MECHE) Study ([www.ucd.ie/jingo/](http://www.ucd.ie/jingo/)) recruited healthy volunteers ($N = 200$, age 18–60, European Ancestry) to a protocol at University College Dublin (UCD), Ireland. Subjects with complete data were analyzed for this study. Subjects completed one or both of two metabolic challenges; an oral lipid tolerance test ($N = 120$; 54 g Fat, 16 g Glucose, 550 kcal) and an oral glucose tolerance test ($N = 129$; 75 g glucose bolus) as described [15,16]. Serial blood samples were taken for measurement of glucose, insulin and cytokines. Habitual diet was assessed using the validated EPIC-Norfolk 131-item food frequency questionnaire (FFQ) [17] and analyzed using the Compositional Analyses from Frequency Estimates (CAFE) program [18]. Ethical approval was obtained from the Research Ethics Committees in UCD. All subjects provided written informed consent. MECHE was registered under NCT01172951 at Clinicaltrials.gov.

**NHANES:** Publicly available data from the National Health and Nutrition Examination Survey (NHANES) 2005-2006 cohort was downloaded from [http://www.cdc.gov/nchs/nhanes/search/nhanes05_06.aspx](http://www.cdc.gov/nchs/nhanes/search/nhanes05_06.aspx). The NHANES FFQ questionnaire was developed by the NIH National Cancer Institute (NCI), and based on the widely-used 124-item NCI Diet History Questionnaire [19]. Data were analyzed and daily food frequency estimates produced using the NCI’s Diet “Calc software. Variables analyzed for the present study included dietary soy intake from FFQ, fasting glucose and insulin, 2-hour glucose post-OGTT, CRP, and demographic information (age, gender, race, BMI). Complete data for $N = 638$ EA and $N = 246$ AA subjects were analyzed here.

**Statistical analysis**

We used linear regression to identify isoflavones as dietary predictors of the inflammatory cytokine response to LPS. To facilitate interpretation, individuals were grouped into moderate-high and low-no soy consumers based on tertiles of intake in GENE. Variables were assessed for normal distribution prior to analysis, and non-normally distributed variables analyzed by non-parametric tests. Group differences were analyzed by Kruskal–Wallis and Mann–Whitney $U$ tests, or analysis of covariance (ANCOVA), adjusting for age, race, gender, BMI. Supplementary linear models in GENE adjusted for dietary macronutrients, micronutrients, vitamins, minerals and baseline CRP. Data were analyzed using SPSS 19 (IBM, Aramonk, NY). Values are presented as means, and standard deviation (SD).

**Results**

**Dietary isoflavone intake is a predictor of inflammatory response in the GENE study**

Baseline anthropomorphic and inflammatory characteristics for GENE Study subjects ($N = 215$, 50% female, 32% AA, 68% EA) are presented in Table 1A, stratified by the upper vs. lower 2 tertiles of isoflavone intake. Dietary composition is presented in Table 1B.

Using multiple linear regression models, we assessed the relationship between macronutrients (carbohydrate, protein, saturated, monounsaturated and polyunsaturated fatty acids) as well as other bioactive dietary nutrients (beta-carotene, biochanin A, choline, coumestrol, daidzein, genistein, glycitein, lycopene, lutein) on peak cytokine responses (TNFα, IL-1RA, IL-6) post LPS. Additional models included all measured dietary variables. In all models employed, the three isoflavones, genistein, daidzein and glycitein emerged as the only significant predictors of peak cytokine response (e.g. $P < 0.001$, 0.005, 0.05 respectively for peak TNFα, and similar for IL-1RA and IL-6). Intake of the three isoflavones was highly inter-correlated ($r^2 = 0.95-0.99$, $P < 0.0001$), and thus the sum of total isoflavone intake was used in subsequent analyses ($P = 0.0038$). The effect of isoflavones on inflammatory response remained significant after Bonferroni correction for the number of independent tests ($P < 0.0042$). There was no association between isoflavone intake and body composition, or baseline levels of cytokines. However there was a significant difference in baseline CRP (1.6 vs. 0.84 mg/dL CRP for low vs. moderate soy consumers, $P = 0.011$ Mann–Whitney U non-parametric test). We ran secondary
Dietary isoflavone intake is associated with evoked responses

models to test for effects of potential covariates (e.g. race and SFA intake differed between soy groups); the association between total isoflavone intake and cytokine responses (TNFα, IL-1RA and IL-6) remained significant in models adjusted for age, gender, race and BMI, as well as in fully adjusted models which additionally adjusted for macronutrient and alcohol intake.

**Moderate isoflavone intake is associated with a more robust cytokine response in GENE**

Isoflavone intake ranged from 0 to 80.47 mg/day, with median intake of 0.45 mg/day. To facilitate further analysis and interpretation, we divided subjects into tertiles of isoflavone intake. We designated individuals in the highest tertile (≥1.48 mg/day total isoflavones, N = 65) as “moderate-high consumers”, and subjects in the lower 2 tertiles as “low-no consumers”. Soy food (tofu, tempeh, soy milk, miso) were the primary sources of isoflavones. We stress that the individuals in the highest tertile did not have a high intake of soy — the cut-point of 1.48 mg/day isoflavone equates to an intake of approximately one portion of soy food a week. As shown in Fig. 2A, individuals with higher isoflavone intake had a significantly higher peak TNFα response (44 vs. 64 fold increase, P = 0.018) compared with individuals consuming low or no amounts. This trend was also observed for IL-1RA (Fig. 2B, 368 vs. 447 fold, P = 0.048) and IL-6 (Fig. 2C, 74 vs. 97 fold, P = 0.2).

| Table 1 | Baseline characteristics and dietary macronutrient composition of soy consumers and soy non-consumers in the GENE Study. EA participants had a higher proportion of soy consumers compared with AA. Dietary macronutrient composition was similar in soy consumers and non-consumers, however non-consumers had a lower intake of saturated fat. |
|-----------------------------------------------|-------------------------------------------------|---------------------|
| **A. Baseline characteristics**              | **Low consumer (isoflavone ≤ 1.48)** | **Moderate consumer (isoflavone > 1.48)** |
| Age (years)                                    | 25.5 (6.5)                                     | 26.0 (5.9)          | 0.174 |
| Gender (male/female)                           | 78/72                                          | 32/33               | 0.711 |
| Race (EA/AA)                                   | 94/56                                          | 53/12               | 0.006 |
| BMI (kg/m²)                                    | 23.9 (2.9)                                     | 23.2 (2.6)          | 0.118 |
| C-reactive protein (mg/dL)                    | 1.6 (3.9)                                      | 0.84 (1.4)          | 0.01  |
| TNFα (pg/ml) baseline                          | 1.3 (0.7)                                      | 1.3 (0.8)           | 0.955 |
| TNFα (pg/ml) peak                              | 45.4 (41.3)                                    | 63.8 (50.8)         | 0.018 |
| IL-1RA (pg/ml) baseline                        | 135.9 (99.6)                                   | 123.0 (50.9)        | 0.909 |
| IL-1RA (pg/ml) peak                            | 39,700 (27,816)                               | 49,030 (31,840)     | 0.048 |
| IL-6 (pg/ml) baseline                          | 2.9 (1.9)                                      | 2.5 (1.6)           | 0.092 |
| IL-6 (pg/ml) peak                              | 160.6 (144.1)                                  | 208.2 (194.2)       | 0.228 |
| **B. Dietary composition (% of total calories)** | **Low consumer (isoflavone ≤ 1.48)** | **Moderate consumer (isoflavone > 1.48)** |
| SFA (%)                                        | 11.0 (3.1)                                     | 9.7 (3.2)           | 0.013 |
| MUFA (%)                                       | 11.2 (2.6)                                     | 11.2 (3.0)          | 0.909 |
| PUFA (%)                                       | 6.3 (2.0)                                      | 6.8 (1.9)           | 0.076 |
| Carbohydrate (%)                               | 51.2 (8.5)                                     | 53.3 (8.9)          | 0.148 |
| Protein (%)                                    | 17.3 (3.9)                                     | 16.9 (3.9)          | 0.361 |
| Alcohol (%)                                    | 1.5 (2.4)                                      | 2.0 (2.9)           | 0.142 |
| Genistien (mg/day)                             | 0.18 (0.17)                                    | 6.9 (7.8)           | <0.0001 |
| Daidzein (mg/day)                              | 0.19 (0.19)                                    | 8.3 (9.3)           | <0.0001 |
| Glycitein (mg/day)                             | 0.02 (0.04)                                    | 1.7 (1.9)           | <0.0001 |
| Total isoflavone (mg/day)                      | 0.39 (0.39)                                    | 16.9 (18.9)         | <0.0001 |

EA: European Ancestry; AA: African Ancestry; BMI, body mass index; IL-6, interleukin 6; TNFα, tumor necrosis alpha; IL-1RA, interleukin 1 receptor agonist; SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids.

Soy consumption is associated with increased inflammatory response to oral lipid load in an independent study (MECHE)

We sought additional independent studies to examine effects of isoflavones on other evoked phenotypes. As calculated isoflavone intake was not available in other studies, we used soy food consumption as a surrogate of isoflavone intake. The Metabolic Challenge (MECHE) Study [15,16] administered an oral lipid challenge to 120 healthy subjects. We hypothesized that postprandial metabolic endotoxemia in response to a lipid load would differ by habitual soy intake. Subjects were dichotomized into soy consumers (N = 20) and soy non-consumers (N = 100) based on their reported intake of soy foods in the FFQ [18]. Soy consumers had significantly higher plasma interferon gamma (IFNγ) 4-hours post-OLTT (P = 0.048), with a significantly greater change in IFNγ response to OLTT (P = 0.001) (Supplementary Fig. 1). This thus provided indirect support for the role of soy isoflavones in the inflammatory response to endotoxemia.

**Dietary isoflavone intake is associated with protection against inflammation-induced insulin resistance in GENE**

We have previously demonstrated that LPS increases markers of insulin resistance in healthy individuals [3]. We examined markers of insulin sensitivity obtained from
The relationship between soy intake and glucose homeostasis is validated in independent samples (MECHE and NHANES)

We further examined the effects of soy intake on glucose homeostasis using OGGT data from the MECHE Study, as well as the US-based National Health and Nutrition Examination Survey (NHANES). In MECHE, we observed a significant difference in the insulin response to glucose load, with lower AUC for insulin in soy consumers ($N = 20$) compared with non-consumers ($N = 109$) (Fig. 3A, $P = 0.03$) despite very similar glucose curves (Fig. 3B), suggesting more efficient insulin-mediated glucose disposal. We examined fasting insulin and glucose, and 2-hour glucose post-OGGT in the NHANES 2005–2006 sample. EA soy consumers had significantly lower fasting insulin and tended towards lower fasting glucose compared with soy non-consumers (Insulin $P = 0.028$; Glucose $P = 0.091$), as well as significantly lower glucose levels post-OGGT ($P < 0.0001$) (Fig. 4A and B). Further, EA soy consumers had significantly lower baseline CRP than non-consumers (0.55 vs. 0.36 mg/dL CRP, $P < 0.001$ Mann—Whitney U). We did not observe any differences in glucose traits or CRP in NHANES AA subjects (Supplementary Fig. 3A,B).

Discussion

We investigated the effects of dietary isoflavone consumption on evoked phenotypes of relevance to inflammatory cardiometabolic disease. Isoflavone consumption was associated with an increased cytokine response to evoked endotoxemia in young healthy subjects and an increased inflammatory response to an oral lipid load in a general population sample. Isoflavone consumers were somewhat protected against inflammation-induced insulin resistance, and soy consumers had lower fasting glucose and insulin, as well as improved glucose homeostasis in response to an oral glucose load. Consistent effects highlighting a role for soy/isoflavone consumption in inflammation and insulin resistance were observed across the three independent samples.

Evoked endotoxemia has been used extensively as a powerful experimental model of inflammatory cardiometabolic disease [3,4], with a low dose approximating the inflammatory state seen in chronic metabolic disease. A higher inflammatory response to experimental LPS may be indicative of enhanced acute immune responses and rapid resolution of infection. Thus, a phenotype characterized by lower resting levels of inflammation, coupled with higher response to inflammatory provocation, may be protective. Meals high in fat, or fat and simple carbohydrates, are known to induce metabolic endotoxemia [6], characterized by increased circulating markers of inflammation, hypothesized to be linked to transient bacteremia from reduced gut barrier function [20]. Dietary factors that improve gut barrier function and protect against metabolic endotoxemia may improve the capacity to mount an effective immune response to pathogens. Isoflavones have

Figure 2 The cytokine response to LPS in individuals with moderate-high ($>1.48$ mg/day, $N = 65$) or low-no ($<1.48$ mg/day, $N = 150$) intake of dietary isoflavones in GENE. Individuals with moderate-high isoflavone intake had a higher inflammatory response across multiple cytokines post-LPS, as measured by peak TNFα levels ($A$, $P = 0.009$); peak IL-1RA levels ($B$, $P = 0.09$) and peak IL-6 levels ($C$, $P = 0.2$). $P$ Value from ANCOVA analysis adjusted for age, sex, race, BMI.

FSIGTT’s at baseline, and 24 h after LPS in a subset of GENE study participants ($N = 118$). There were no baseline differences in insulin sensitivity ($S_i$) between the moderate and low isoflavone consumers. Following LPS, $S_i$ decreased in both groups, however there was a significant difference in $S_i$ post-LPS ($P = 0.008$), with moderate consumers ($N = 40$) experiencing less of a decrease in $S_i$ than low consumers ($N = 78$), suggesting that soy intake may be protective against inflammation-induced insulin resistance (Supplementary Fig. 2). This effect was stronger in EA individuals, with no effect in AA alone; however due to small sample size ($N = 30$ AA) and low numbers of AA soy-consumers ($N = 3$), we lacked power to assess race differences in the GENE FSIGTT sample.
been shown to improve intestinal barrier integrity [21] and reduce colitis in animal models [22], potentially through modulation of the microbiome [23]. High soy consumption has been associated with reduced levels of circulating cytokines in women [24,25]. Thus, regular consumption of soy foods may confer protection against diet-induced metabolic dysfunction, and reduce the development of insulin resistance. We observed a significantly increased IFNγ response to oral lipid load in soy consumers in the MECHE study, indicative of greater immune response, suggesting that soy consumption may have an effect on metabolic endotoxemia. Although there are no official dietary recommendations relating to isoflavone intake, intake of 40–80 mg/day have been suggested for cancer prevention or modulation of lipoproteins in hyperlipidemia [26]. We note that we did not observe any differences in cholesterol (total, LDL or HDL) or triglycerides by isoflavone consumption in GENE. Our findings, while not informing ideal dosage, suggest that relatively low intake of isoflavones may be sufficient to obtain benefits in healthy individuals. In support of this, habitual soy intake in healthy US women was associated with lower CRP, with comparable intakes (median intake 0.55 mg.day compared with 0.45 mg/day in our study) [25], highlighting the potential value of “low” intake.

Elevated fasting levels of glucose and insulin, as well as increased AUC following OGTT are risk factors for development of insulin resistance, T2D and atherosclerosis [27]. We observed interesting effects of soy consumption on markers of glucose homeostasis and insulin resistance in the GENE, MECHE and NHANES study samples. Soy consumption 1) conferred protection

**Figure 3** Insulin response to Oral Glucose Tolerance Test (OGTT) differs by soy consumption in MECHE. In the MECHE study, soy consumers (N = 20) had a significantly lower overall insulin response to glucose bolus during OGTT (A) (ΔAUC P = 0.03), despite similar glucose curves (B) to soy non-consumers (N = 109).

**Figure 4** Fasting insulin and glucose, and 2-hour glucose post-OGTT in NHANES are lower in EA soy consumers than soy non-consumers. Fasting insulin was significantly lower in soy consumers (A, P = 0.028 N = 121 consumer, N = 517 non-consumer), while fasting glucose trended towards being lower (B, P = 0.091) and glucose 2-hours post-OGTT was significantly lower (C, P < 0.0001).
against LPS-induced decline in insulin sensitivity; 2) was associated with improved insulin-mediated glucose disposal as evidenced by reduced insulin secretion required to normalize glucose post-OGTT in MECHO, and 3) was associated with lower fasting insulin and lower 2-h glucose post-OGTT in NHANES. We did not observe differences in AA. Race differences in diabetes prevalence and glucose homeostasis are known [28], and as-yet poorly understood race differences in immune response and maintenance of normoglycemia may explain the failure of soy consumption to modulate parameters of insulin sensitivity in AA. However, smaller sample size, and lower levels of soy consumption may have hampered the ability to detect effects of soy consumption in AA. The soy isoﬂavone genistein has been found to protect against cytokine-mediated dysfunction of pancreatic beta cells [29], while daidzein has been found to promote glucose uptake and reduce cytokine expression [30], suggesting that soy consumption may protect against inﬂammation-induced insulin resistance in humans through modulation of cytokine signaling [31].

Our study had unique strengths. To our knowledge, this is the first study to look at the association of habitual soy food or isoﬂavone consumption on multiple evoked phenotypes in humans, including inﬂammatory responses in the setting of evoked endotoxemia, as well as metabolic and insulin responses to high lipid or sugar meals. As reported by our group and others, an evoked phenotype model allows for improved ability to investigate dynamic data [4,32–34], and this may be particularly important in nutritional investigations. This study also had certain limitations; no dietary soy supplementation was implemented, and we relied on self-reported estimates of isoﬂavone/soy intake in all studies. While 3-day food records and FFQs are validated and widely-used dietary assessment methods, all self-reported data is subject to bias [35]. We did not have estimates of isoﬂavone consumption in the 2 supporting studies, and thus relied on soy food intake as a surrogate. Further, we cannot exclude a role for other ﬂavonoids or phytonutrients [36]. Soy/isoﬂavone intake was generally low, as expected in North American/European populations, but was comparable to the intake recently shown to be associated with lower CRP in healthy women [25]. Despite the limitations, the present study demonstrated a signiﬁcant effect of moderate soy/isoﬂavone consumption on metabolic health. These effects are seen with low isoﬂavone intake, equivalent to approximately one portion a week of isoﬂavone-rich soy food. Thus, these intakes are very achievable, even in populations without a high traditional intake of soy food. Measuring evoked phenotypes may be more informative than describing resting characteristics, and may increase power to detect effects of nutrition on biomarkers of cardiometabolic health. Future intervention studies, designed to assess the effect of soy/isoﬂavone supplementation on evoked phenotypes in both healthy and disease populations of different ancestry, may help to determine subsets of individuals who would beneﬁt from increased soy consumption.

Funding

The GENE Study was supported by the National Center for Research Resources and the National Center for Advancing Translational Sciences, National Institutes of Health, through Grant UL1TR000003 as well as a NIH-NHLBI SCCOR Project grant (P50-HL-083799) to MPR. JFF is supported by a postdoctoral fellowship grant from the American Heart Association (12POST11840017). MPR is also supported by R01-HL-111694, R01-HL-113147, R01-DK-090505, U01-HL-108636 and K24-HL-107643. HMR is supported by SFI PI Programme (11/PI/1119). The content is solely the responsibility of the authors and does not necessarily represent the ofﬁcial views of the NIH. The MECHO Study was funded under the Food for Health Research Initiative (NDP 2007-2012; 07FHRUCD1) by the Irish Department of Agriculture, Food & the Marine.

Authors’ contributions to manuscript

JFF and MPR designed the research. JFF conducted research in the GENE Study. MFR, ERG, LB and HMR conducted research in the MECHO Study. JFF analyzed data and wrote the paper. All authors read and approved the final manuscript.

Conflict of interest

The authors have no conﬂicts of interest to disclose.

Acknowledgments

We would like to thank the participants of the GENE, MECHO, and NHANES studies, the staff at the UPenn Clinical Translational Research Center and the support of the UPenn Nutrition Core Dietary Assessment Unit.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.numecd.2014.03.010.

References
