Increased Lean Red Meat Intake Does Not Elevate Markers of Oxidative Stress and Inflammation in Humans[1]

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Abstract

Red meat intake has been associated with increased risk of coronary heart disease and type 2 diabetes, but it remains uncertain whether these associations are causally related to unprocessed lean red meat. It has been proposed that iron derived from red meat may increase iron stores and initiate oxidative damage and inflammation. We aimed to determine whether an increase in unprocessed lean red meat intake, partially replacing carbohydrate-rich foods, adversely influences markers of oxidative stress and inflammation. Sixty participants completed an 8-wk parallel-designed study. They were randomized to maintain their usual diet (control) or to partially replace energy from carbohydrate-rich foods with ~200 g/d of lean red meat (red meat) in isoenergetic diets. Markers of oxidative stress and inflammation were measured at baseline and at the end of intervention. Results are presented as the mean between-group difference in change and [95% CI]. Red meat, relative to control, resulted in: higher protein [5.3 (3.7, 6.9) % of energy], lower carbohydrate [−5.3 (−7.9, −2.7) % of energy], and higher iron [3.2 (1.1, 5.4) mg/d] intakes; lower urinary F_2-isoprostane excretion [−137 (−264, −9) pmol/mmol creatinine], lower leukocyte [−0.51 (−0.99, −0.02) $\times 10^9$/L] counts, and a trend for lower serum C-reactive protein concentrations [−1.6 (−3.3, 0.0) mg/L, $P = 0.06$]; and no differences in concentrations of plasma F_2-isoprostanes [−12 (−122, 100) pmol/L], serum γ-glytamyltransferase [−0.8 (−3.2, 1.5) U/L], serum amyloid A protein [−1.4 (−3.4, 0.5) mg/L], and plasma fibrinogen concentrations [−0.08 (−0.40. 0.24) g/L]. Our results suggest that partial replacement of dietary carbohydrate with protein from lean red meat does not elevate oxidative stress or inflammation. J. Nutr. 137: 363–367, 2007.

Introduction

Accumulating evidence suggests that partial replacement of refined carbohydrates with low-fat sources of protein may benefit weight loss and risk of cardiovascular disease (1). Within many populations, advice to increase protein intake, at the expense of carbohydrates, could result in an increase in red meat intake. However, there is continuing concern about the health effects of red meat. Population studies have linked higher red meat intake with increased risk of coronary heart disease (2,3) and type 2 diabetes (4–6), but it remains uncertain whether these associations are causally related to unprocessed and lean red meat. Intake of saturated fat, which is often tied to red meat intake within populations (2,7,8), and processing of red meat (4–6) may at least partly account for these associations. Effects of iron derived from red meat to increase iron stores and initiate oxidative damage and inflammation is another proposed pathway (5,9,10). An increase in heme-iron from red meat may result in an increase in the availability of iron capable of enhancing the generation of free radicals in the body. However, as yet there is little support for this proposal from results of human intervention trials.

Iron is a redox-active transition metal that may contribute to production of reactive oxygen species, oxidative stress, and inflammation (11). Oxidative stress (12) and inflammation (13) are important contributors to the pathogenesis of vascular disease. Higher iron intake has been associated with increased risk of coronary heart disease (14) and type 2 diabetes (15) and markers of body iron stores have been positively associated with risk of type 2 diabetes (16). However, blood donations, resulting in reduced iron stores, are not associated with lower risk of heart disease (17) or type 2 diabetes (16). Furthermore, although red meat and heme-iron intake have often been positively associated with markers of iron stores in population studies (18–20), changing red meat intake in individuals with normal iron status may not increase markers of iron stores (21,22). The effects of changes in red meat intake in the intervention setting on markers of oxidative stress and inflammation are uncertain.

Therefore, our objective was to determine whether an increase in unprocessed and lean red meat intake, partially replacing carbohydrate-rich foods, adversely influences markers of oxidative stress and inflammation. Markers of iron status have also been assessed and their relations with any effects of an

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increase in red meat intake on markers of oxidative stress and inflammation have been investigated.

Materials and Methods

Participants. Nonsmoking men and women >20 y old with elevated blood pressure were recruited from the general population. The details of all inclusion and exclusion criteria, and effects on blood pressure (the primary outcome measurement in this trial), blood lipids, glucose, and insulin concentrations have been reported previously (23). Seventy-one individuals were randomized, 36 in the control group and 35 in the intervention group, and 60 participants completed the study, 31 in the control group and 29 in the intervention group. The study was approved by the Royal Perth Hospital Human Ethics Committee and all participants gave written informed consent prior to inclusion in the study. All procedures followed were in accordance with institutional guidelines.

Study design. In a parallel-designed study, participants were randomly assigned, using computer generated random numbers, to either maintain their usual diet (control) or partially replace energy from carbohydrate with protein from lean red meat (red meat) for 8 wk. Participants in the red meat group were supplied with lean red meat: ~215 g/d raw weight, depending on usual energy intake. They were instructed to consume the meat in place of carbohydrate-rich foods including bread, pasta, rice, potatoes, and breakfast cereals. The objective in the red meat group was to achieve an ~35–40 g/d (7–8% of total energy intake) higher protein intake compared with the control group.

Dietary and lifestyle assessments. A 3-d weighed food diary completed at baseline and during the last week of intervention was analyzed using FoodWorks Software (Xyris) based on the Australian Food Composition Database. Height was measured at baseline, and body wt was measured at baseline and end of intervention. Food and alcohol intake, physical activity, health status, and medication were monitored by interview with a dietitian at each of the 4 biweekly visits.

Biochemistry and hematology. All measurements were performed at baseline and end of intervention. PathWest Laboratory Medicine WA, Royal Perth Hospital performed all routine biochemical and hematological measurements. Markers of iron status included serum ferritin, transferrin saturation, and iron concentrations. Markers of oxidative stress included serum γ-glytamyltransferase (GGT)[3] and plasma and urinary concentrations of F_2-isoprostanes. Markers of inflammation included white cell counts (leucocytes, neutrophils, lymphocytes, and monocytes), high sensitivity plasma C-reactive protein concentrations (HS-CRP), serum amyloid A protein (SAA), and plasma fibrinogen concentrations. Markers of iron status and GGT were measured in serum on the Hitachi 917 analyzer (Roche Diagnostics). Iron was measured using a colorimetric assay with an observed intra-assay CV of <2%. Ferritin and transferrin were determined using an immunoturbidometric assay, with an intra-assay CV of <10% for ferritin and <3% for transferrin. Serum GGT was measured using an enzymatic colorimetric assay with an intra-assay CV of <2.5%. A full blood picture, including absolute cell counts, was performed by optical flow cytometry using a Cell-Dyn 4000 analyzer (Abbott Laboratories). Serum HS-CRP and SAA were measured by particle-enhanced immunonephelometry using a kits produced by Dade Behring on a BN-Systems analyzer. The immunonephelometry is enhanced by using polystyrene particles coated with a monoclonal antibody to CRP or SAA to obtain intra-assay CVs of <7% for each assay. Plasma fibrinogen was measured using the Clauss technique on the STA-R coagulation analyzer (Diagnostica Stago) using bovine thrombin (Dade-Behring). This technique has an intra-assay CV of <5%. Plasma and urinary F_2-isoprostane concentrations were measured by GC-mass spectrometry using a previously described method (24).

Statistics. Statistical analyses were performed using SPSS 11.5 software. Log transformation was performed on GGT, urinary F_2-isoprostane concentrations, and HS-CRP, which were not normally distributed. Baseline and end of intervention data are presented as means ± SD or geometric means [95% CI] for log-transformed variables. Between-group differences in change (end of intervention minus baseline) are presented as means [95% CI]. Differences were considered significant at $P < 0.05$. At baseline, characteristics of participants in the 2 groups were compared using the independent-samples t test. The paired t test was used to assess within-group changes. General linear models were used to assess between-group differences in change and to adjust for potential confounders. Pearson's correlation was used to investigate the relations between changes in markers of iron status and changes in markers of oxidative damage and inflammation within the red meat group.

Results

Baseline characteristics. Sixty participants completed the study. There were 31 participants (18 men and 13 women) in the control group aged 60.0 ± 9.9 y with BMI of 27.9 ± 4.0 kg/m^2. There were 29 participants (20 men and 9 women) in the red meat group aged 57.2 ± 7.3 y with BMI of 27.5 ± 3.1 kg/m^2. BMI did not differ between groups at baseline and did not change during the study.

Energy and nutrient intakes. Energy and nutrient intakes did not differ between groups at baseline (data not shown). Participants complied with the set dietary changes. Intake of lean red meat increased in the red meat group by ~200 g raw meat/d. In comparison to the control group, there was a higher protein (mean difference in change [95% CI]: 5.3 [3.7, 6.9]% of energy; 36 [26,46] g/d), lower carbohydrate (primarily starch) [−5.3 (−7.9, −2.7)% of energy] and higher iron [3.2 (1.1, 5.4) mg/d] intake.

Markers of iron status. The groups did not differ in markers of iron status at baseline (**Table 1**). Hemoglobin concentrations tended to increase [2 (0, 4) g/L, $P = 0.07$] and erythrocyte counts increased [0.08 (0.01, 0.15) × 10^{12}/L, $P = 0.03$] from baseline in the red meat group. However, the change in hemoglobin concentrations and erythrocyte counts did not differ between groups. In comparison to the usual diet (control), partial replacement of dietary carbohydrate for protein in the form of lean red meat (red meat) did not increase any of the markers of iron status. On the contrary, in the red meat group serum iron concentrations and serum transferrin saturation were significantly lowered from baseline and in comparison to control. Serum ferritin concentrations were not affected by the diets (Table 1).

Markers of oxidative stress. Urinary and plasma F_2-isoprostane and GGT concentrations did not differ at baseline (**Table 2**). At baseline, plasma and urinary F_2-isoprostane concentrations were correlated ($r = 0.34$, $P = 0.01$). Red meat resulted in lower urinary but not plasma F_2-isoprostane concentrations, relative to the control group (analyzed as mean between-group difference in change; Table 2). There was a decrease in GGT from baseline to end of intervention within the red meat group [−1.9 (−3.2, −0.5) U/L, $P = 0.009$], but red meat did not change GGT relative to control (Table 2). At baseline, GGT was correlated with alcohol intake ($r = 0.29$, $P = 0.03$), but the magnitude of the between-group difference in GGT was not altered after adjustment for either baseline alcohol intake or change in alcohol intake. Within the red meat group, the change in urinary

[3] Abbreviations used: GGT, γ-glytamyltransferase; HS-CRP, high sensitivity C-reactive protein; SAA, serum amyloid A protein.

TABLE 1 Markers of iron status at baseline and end of intervention (post) and between-group differences in men and women randomized to maintain their usual diet (control group) or partially replace dietary carbohydrate with protein from lean red meat (red meat group)

Variable	Control group, $n = 31$	Red meat group, $n = 29$
Baseline serum ferritin,[1] *μg/L*	133 ± 112	158 ± 128
Post	132 ± 111	140 ± 100
Difference[2]	−17 (−39, 5)	
Baseline serum iron, *μmol/L*	19.2 ± 6.9	17.9 ± 4.5
Post	20.5 ± 7.3	15.8 ± 3.9 *
Difference	−3.4 (−6.2, −0.6) **	
Baseline serum transferrin, *μmol/L*	32.9 ± 4.1	31.4 ± 3.3
Post	33.1 ± 4.1	31.7 ± 3.5
Difference	0.2 (−0.9, 1.3)	
Baseline transferrin saturation, *%*	29.7 ± 11.5	28.9 ± 8.2
Post	31.3 ± 11.9	25.3 ± 7.2 *
Difference	−5.2 (−9.6, −0.7) **	
Baseline hemoglobin, *g/L*	137 ± 12	136 ± 10
Post	137 ± 12	138 ± 9
Difference	2 (−1, 5)	
Baseline erythrocyte count, $\times 10^{12}$/L	4.43 ± 0.39	4.43 ± 0.30
Post	4.46 ± 0.43	4.50 ± 0.32 *
Difference	0.05 (−0.06, 0.15)	

[1] Baseline and post values are mean ± SD. * $P < 0.05$ (paired *t* test).
[2] Differences are mean differences between the change in the red meat group, from baseline to post, and the change in the control group [95% CI]. ** $P < 0.05$ (general linear models).

TABLE 2 Markers of oxidative stress and inflammation at baseline and end of intervention (post) and between-group differences in men and women randomized to maintain their usual diet (control group) or partially replace dietary carbohydrate with protein from lean red meat (red meat group)

Variable	Control group, $n = 31$	Red meat group, $n = 29$
Baseline urinary F_2 isoprostanes,[1] *pmol/mmol creatinine*	873 (735, 1033)	812 (665, 925)
Post	916 (750, 1119)	735 (645,871)
Difference[2]	−137 (−264, −9) **	
Baseline plasma F_2-isoprostanes, *pmol/L*	1392 ± 245	1406 ± 333
Post	1362 ± 280	1364 ± 270
Difference	−12 (−122, 100)	
Baseline serum GGT, *U/L*	20.5 (17.1, 24.6)	22.8 (19.0, 27.4)
Post	19.8 (16.7, 23.5)	21.1 (17.7, 25.3) *
Difference	−0.8 (−3.2, 1.5)	
Baseline leukocyte count, $\times 10^9$/L	5.79 ± 1.23	5.68 ± 1.28
Post	5.87 ± 1.19	5.26 ± 1.14 *
Difference	−0.51 (−0.99, −0.02) **	
Baseline neutrophil count, $\times 10^9$/L	3.46 ± 0.96	3.33 ± 0.98
Post	3.52 ± 1.00	3.04 ± 0.80
Difference	−0.35 (−0.85, 0.15)	
Baseline lymphocyte count, $\times 10^9$/L	1.62 ± 0.49	1.68 ± 0.34
Post	1.73 ± 0.59 *	1.60 ± 0.45
Difference	−0.20 (−0.36, −0.05) **	
Baseline monocyte count, $\times 10^9$/L	0.50 ± 0.13	0.48 ± 0.15
Post	0.49 ± 0.13	0.44 ± 0.11
Difference	−0.04 (−0.10, 0.02)	
Baseline serum HS-CRP, *mg/L*	1.36 (0.90, 2.06)	1.65 (1.20, 2.25)
Post	1.59 (1.00, 2.52)	1.30 (0.95, 1.78)
Difference	−1.6 (−3.3, 0.0)	
Baseline SAA, *mg/L*	3.8 (2.9, 5.0)	4.1 (2.9, 5.7)
Post	3.9 (3.0, 5.2)	3.5 (2.6, 4.6) *
Difference	−1.4 (−3.4, 0.5)	
Baseline plasma fibrinogen, *g/L*	3.2 ± 0.7	3.2 ± 0.7
Post	3.3 ± 0.7	3.1 ± 0.6
Difference	−0.08 (−0.40, 0.24)	

[1] Baseline and post values are means ± SD or geometric mean [95% CI] for non-normally distributed variables. * $P < 0.05$ (paired *t* test).
[2] Differences are mean differences between the change in the red meat group, from baseline to post, and the change in the control group [95% CI]. ** $P < 0.05$ (general linear models).

F_2-isoprostane concentrations was positively correlated with change in serum iron ($r = 0.44$, $P = 0.02$) and change in transferrin saturation ($r = 0.44$, $P = 0.02$).

Markers of inflammation. White cell counts, plasma HS-CRP, SAA, and plasma fibrinogen concentrations did not differ at baseline (Table 2). Within the red meat group, there was a decrease in leukocyte counts [−0.42 (−0.81, −0.02) $\times 10^9$/L, $P = 0.04$] and SAA [−1.3 (−2.3, −0.2) mg/L, $P = 0.02$] from baseline to end of intervention. Relative to control (analyzed as mean between-group difference in change; Table 2), red meat resulted in lower leukocyte counts and a trend for lower HS-CRP ($P = 0.06$). Plasma fibrinogen concentrations were not altered (Table 2). Within the red meat group, the change in leukocyte count was positively correlated with change in serum ferritin ($r = 0.45$, $P = 0.01$).

Discussion

We have investigated whether an increase in the intake of lean red meat, at the expense of carbohydrate-rich foods, adversely influences markers of oxidative stress and inflammation. Population studies have related red meat and iron intake to increased risk of heart disease and type 2 diabetes. However, there is little direct evidence that these associations are causally related to red meat protein per se or to iron. The results of intervention studies with lean red meat suggest no adverse effects on blood cholesterol concentrations, markers of thrombosis (25), blood pressure (23), and oxidative stress and inflammation.

Red meat is a source of highly bioavailable heme-iron (26,27). Dietary heme-iron can significantly increase iron absorption at a single meal (28). In our study, there was an increase in total and heme-iron intake from the red meat. Therefore, the suggestion of reduced iron status with an increase in red meat and iron intake was unexpected. However, in most people, there is effective control of iron absorption, preventing iron overload (29). Ongoing consumption of a diet with higher iron bioavailability often does not alter markers of iron stores such as ferritin in iron-replete individuals (22,30). In addition, a previous 7-wk intervention study has also shown that an increase in meat and iron intake resulted in lower ferritin concentrations and transferrin saturation (21). The reasons for these changes in markers of iron status, which are suggestive of reduced iron status, are not clear. It is possible that differences in protein and carbohydrate intake may have influenced long-term iron bioavailability (21). It is also likely that markers of body iron pools such as ferritin, serum iron, and transferrin saturation do not always provide an accurate indication of total body iron

stores (31). Furthermore, in this study, changes in markers of body iron pools may not be sensitive indicators of changes in iron intake or total body iron stores.

Iron-derived reactive oxygen species have been implicated in the pathogenesis of vascular disease (32). It is suggested that iron can contribute to oxidative stress and inflammation (11,32,33) and that oxidative stress (34) and inflammation (35) are risk factors for diabetes and heart disease. However, reactive oxygen species are produced by free, but not bound, iron (36) and the body has evolved a metabolic system that minimizes the availability of free iron (32). In addition, markers of iron status such as ferritin, transferrin, and transferrin saturation may not reflect the availability of iron free to be involved in reactive oxygen species production (37).

To date, there is limited direct evidence for effects of red meat or iron supplementation to influence oxidative stress in vivo in humans. Circulating GGT has been proposed to be a nonspecific marker of oxidative stress (38). In population studies, GGT has been associated with risk factors for cardiovascular disease (39), type 2 diabetes (39), and mortality from cardiovascular disease (40). A major dietary determinant of GGT is alcohol, but alcohol intake is unlikely to account for the observed relations of GGT with diabetes and cardiovascular disease (10,39). Red meat and heme-iron intake have been associated with GGT and it was suggested that this association was consistent with a link between heme-iron and oxidative stress (10). However, data from human intervention trials are needed to support this suggestion. Our data, which showed that a modest increase in lean red meat and heme-iron intake does not increase GGT, do not support a causal link between red meat and heme-iron intake and raised GGT.

F_2-isoprostanes are formed by the nonenzymatic free radical oxidation of arachidonic acid (41). They reflect oxidative damage to lipoproteins and tissues (41,42) and are currently thought to be one of the best available markers of in vivo lipid peroxidation (43). Iron supplements increase lipid peroxidation in rats (44) and there are data to suggest similar effects in humans (45,46). A small study involving 3 healthy participants found an increase in urinary isoprostanes following supplementation with 120 mg iron/d (46). The effects of a modest increase in the intake of iron from lean red meat on in vivo markers of lipid peroxidation have not previously been investigated. We found that plasma F_2-isoprostane concentrations were not significantly altered, but urinary F_2-isoprostane concentrations were significantly reduced with red meat relative to control. The 2 F_2-isoprostane measurements were significantly correlated, but this is not always demonstrated (47). Plasma F_2-isoprostane concentrations are likely to provide an accurate reflection of oxidative stress in vivo, with the major limitation being the potential for acute fluctuations. This limitation may be largely overcome by measuring F_2-isoprostanes in a 24-h urine sample, but it has been suggested that urinary F_2-isoprostanes may be partly derived from local production in the kidney (48). The apparent discrepancy between our results, suggesting no change or reduced oxidative stress with an increase in iron intake, and results of previous studies (44–46) may relate to the dose of iron provided. The increase in iron intake in our study of ~3 mg/d was small in comparison to previous studies. Our results are not consistent with the suggestion that a modest increase in the intake of heme-iron from red meat increases oxidative stress.

Inflammation appears to be important in the pathogenesis of atherosclerosis leading to coronary heart disease (13,35). Blood leukocyte counts (49), HS-CRP (50), SAA (51), and plasma fibrinogen concentrations (52) provide a useful nonspecific indication of inflammation in vivo. Few studies have investigated the effects of iron supplementation (46,53) or differences in red meat intake (54) on markers of inflammation. Postpartum iron supplementation (80 mg/d) for 12 wk in nonanemic iron-deficient women did not significantly alter CRP or leukocyte counts (53). A small study involving 3 healthy participants found an increase in interleukin-4, but not CRP or leukocyte counts, following supplementation with 120 mg iron/d (46). There was no effect of higher red meat intake, in comparison to carbohydrate, resulting in an approximate 5 mg/d increase in iron intake on HS-CRP in obese women (54). We found that partial replacement of carbohydrate with lean red meat reduced some markers of inflammation and did not significantly alter others. This result is not consistent with suggested proinflammatory effects of an increase in iron intake from lean red meat.

The results of our study suggest decreased rather than increased oxidative stress and inflammation when lean red meat intake is increased at the expense of dietary carbohydrate-rich foods. However, this does not rule out a link between iron status and oxidative stress and inflammation. In fact, the observed reduction in serum iron and transferrin saturation, and the relations of change in urinary F_2-isoprostanes with change in markers of iron stores, would be consistent with such a link. Alternatively, it is possible that changes in both markers of iron status and urinary F_2-isoprostane concentrations may be independent manifestations of the increase in red meat intake and or the decrease in carbohydrate intake.

In conclusion, the results of this study suggest that a modest increase in the intake of lean red meat in iron-replete individuals is unlikely to increase oxidative stress or inflammation. This conclusion is limited to the short term when lean red meat provided to participants partially replaces carbohydrate in the diet. Our results do not support the suggestion that higher red meat intake leads to increased risk of heart disease and type 2 diabetes via effects of iron to increase oxidative stress and inflammation.

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