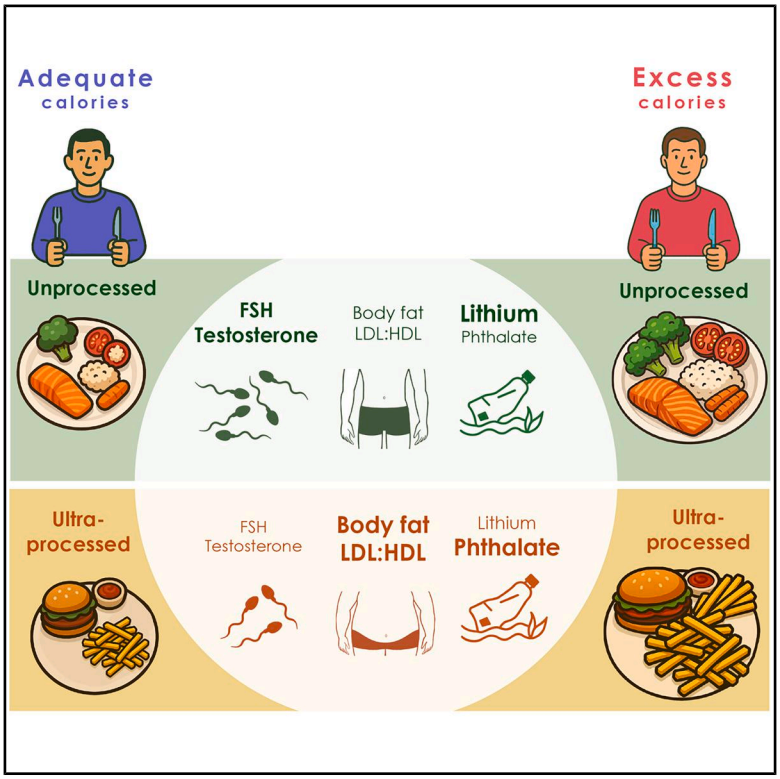
Cell Metabolism
Clinical and Translational Report

Effect of ultra-processed food consumption on male reproductive and metabolic health

Graphical abstract



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In brief

This randomized controlled nutrition intervention conducted in males of reproductive age shows that, compared with an unprocessed diet, consumption of ultra-processed foods impairs metabolic and reproductive health. Effects of ultra-processed foods were independent of caloric load, providing evidence that the ultra-processed nature of food is detrimental to health.

Highlights

- Compared with an unprocessed diet, UPF impaired cardiometabolic and reproductive health
- The deleterious effects of a UPF diet were independent of total caloric intake
- A UPF diet altered the balance of several hormones, including GDF-15 and FSH
- A UPF diet was associated with higher serum concentration of the phthalate cxMINP

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Effect of ultra-processed food consumption on male reproductive and metabolic health

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SUMMARY

Consumption of ultra-processed food is associated with increased caloric intake and impaired health. Here, we conducted a nutrition trial (NCT05368194) with controlled, 2 × 2 crossover design and tested whether ultra-processed food impairs reproductive and metabolic fitness, with further aggravation by excess caloric intake. Comparing the response from an unprocessed to ultra-processed diet identified increased body weight and low-density lipoprotein (LDL):high-density lipoprotein (HDL) ratio, independent of caloric load. Several hormones involved in energy metabolism and spermatogenesis were affected, including decreased levels of growth/differentiation factor 15 and follicle-stimulating hormone. Sperm quality trended toward impairment, with a decrease in total motility. Differential accumulation of pollutants between the discordant diets were detected, such as decreased plasma lithium and a trend for increased levels of the phthalate mono (4-methyl-7-carboxyheptyl)phthalate (cxMINP) in serum, following the ultra-processed diet. Alteration in caloric load alone had distinct effects on the measured outcomes. This study provides evidence that consumption of ultra-processed food is detrimental for cardiometabolic and reproductive outcomes, regardless of excessive caloric intake.

INTRODUCTION

The consumption of ultra-processed foods (UPFs) has markedly increased globally,[1] now accounting for over 50% of total energy intake in the UK,[2] Australia,[3] Canada,[4] and the USA.[5,6] UPFs are NOVA category 4 foods and defined as foods that are industrially processed and composed of highly transformed, derived, or synthesized ingredients.[7] UPFs contain macro- and micronutrients associated with poor metabolic health, including saturated fats, refined carbohydrates, and energy-dense formulations.[8,9] UPFs are suspected to contain contaminants such as plasticizers, including phthalates, which may leach into the food supply through processing or packaging.[10] Phthalate levels are elevated in urine after UPF consumption and may impair metabolic and reproductive function through endocrine hormone disruption.[11–14]

A growing body of cross-sectional and prospective studies has identified the association between increased UPF consumption and elevated risk of chronic diseases, including cardiovascular and metabolic disease, certain cancers, and mental health disorders.[15] Although many studies have demonstrated a link between UPF consumption and clinical markers of impaired cardiometabolic health, such as increased body weight, hypertension, and reduced HDL-cholesterol,[16] fewer have focused on its impact on male reproductive health.[17,18] Concurrent with the rise in UPF consumption, semen quality has declined globally, with sperm count falling by approximately 60% since the 1970s.[19,20] Suspected factors underlying the decrease in semen quality parameters include trends of increased body weight, intake of saturated and trans-fat, and exposure to endocrine-disrupting chemicals from industrial origin, wherein UPF intake is

likely a causal factor for these parameters.[8,11,19,21–23] Despite the simultaneous increase in UPF consumption and decline in global semen quality, it is not clear whether UPF consumption plays a direct role in the deterioration of broader fertility trends.

To date, few randomized controlled studies have examined the effects of an ultra-processed diet on human health.[24,25] These studies demonstrated a causal relationship between UPF consumption and disruptions in clinical parameters associated with cardiometabolic health.[24,25] Participants consuming UPF exhibited an energy intake approximately 500–800 kcal/day higher than those following a controlled unprocessed diet.[24,25] Whether the negative effects of UPF are solely due to the increased caloric intake or a combined effect of increased caloric intake with inherently harmful properties of the UPF itself is unknown.

In the present study, we conducted a dual-arm 2 × 2 crossover-designed dietary intervention to compare the effects of an ultra-processed to an unprocessed diet, wherein participants were randomized to receive both diets at either calorically adequate or excess loads. Diets were provided for 3 weeks, and dietary makeup included an average of 77% of calories from UPFs and 5.5% from unprocessed foods in the ultra-processed diet and 1% of energy from UPFs and 66% from unprocessed foods for the unprocessed diet. Participants received a fixed equal quantity of total calories from both the unprocessed and ultra-processed diets to isolate the specific effects of UPF consumption from those of caloric intake alone. Only secondary outcomes are presented in this report. The primary outcome set in the registered clinical trial, sperm DNA methylation, is not reported here.

RESULTS

Intervention and participant characteristics

Forty-three male participants were randomized and subjected to two distinct diets in a 2 × 2 crossover fashion (Figure 1A). A wash-out period between the respective dietary interventions was included to avoid the influence of previous experimental diet. Participants were provided either an ultra-processed or unprocessed type diet for 3 weeks (see details of the diets in STAR Methods and Table S1) at calorically adequate or excess quantities (Figure 1A). Within each diet group, diets were similar in macronutrient composition (Figure 1B). The ultra-processed diet was designed to reflect a typical ultra-processed diet consumed in nations with heightened UPF intake. Compared with the unprocessed diet, the ultra-processed diet contained elevated levels of saturated fat, cholesterol, refined grains, added sugars, and dairy products and lower amounts of fiber (Figures 1C and 1D; Table S1). A summary of participant characteristics at baseline is presented in Tables 1 and S2. Total caloric consumption while accounting for dietary adherence was similar between diets within each calorie arm (Figures S1A–S1D).

Cardiometabolic health

Consumption of the ultra-processed versus the unprocessed diet led to a 1.4 and 1.3 kg weight increase in the adequate and excess calorie arms, respectively (adequate, 95% confidence interval [CI] [0.57, 2.25]; excess, 95% CI [0.47, 2.08]) (Figure 2A; Table 1). Differences in weight change between dietary groups appear to be primarily driven by a weight loss observed in response to the unprocessed dietary intervention (Table S3). Although the different diets had no effect on lean mass (Figure 2B; Table 1), there was a significant increase in fat mass between the two diets, with approximately 1.00 and 0.96 kg more fat mass following the ultra-processed versus the unprocessed diet in the adequate and excess calorie arms, respectively (adequate, 95% CI [0.41–1.64]; excess, 95% CI [0.38–1.53]) (Figure 2C; Table 1).

Cholesterol levels, measured as total cholesterol and low-density lipoprotein (LDL):high-density lipoprotein (HDL) ratio, were increased in response to the ultra-processed versus unprocessed diet in the adequate calorie arm only (Figures 2D and 2E; Table 1), and diastolic blood pressure was increased in the excess calorie arm only (Figure 2F; Table 1). Changes in cholesterol levels, but not LDL:HDL ratio, appear to be driven by the unprocessed diet in the adequate calorie arm, as suggested by the comparison between baseline and post unprocessed diet levels (Figures 2D and 2E; Table S3). C-peptide was increased in the adequate calorie arm only (Figure 2G; Table 1). Intake of the ultra-processed versus unprocessed diet led to a trend of increased thyroid hormones triiodothyronine (T3) and thyroid-stimulating hormone (TSH) in the adequate calorie group only (Figure 2H; Table 1). In the excess calorie group, growth/differentiation factor 15 (GDF-15) levels were decreased, while leptin trended toward increased levels with the ultra-processed diet (Figures 2I and 2J; Table 1).

Reproductive hormones and function

Compared with the unprocessed diet, consumption of the ultra-processed diet caused a decrease in follicle-stimulating hormone (FSH) levels in the excess calorie arm (Figure 3A; Table 1) and a trend for decreased circulating levels of testosterone in the adequate calorie arm (Figure 3B; Table 1). Sperm quality trended toward a decrease, with reduced total sperm motility in the excess calorie arm (Figure 3C; Table 1). Sperm concentration was not significantly changed (Figure 3D; Table 1).

Altered toxin levels and inflammatory response

Endocrine-disrupting compounds (EDCs) may impair reproductive function and perturb sex hormone secretion.[26] To gain insight into the association between EDCs and reproductive function after UPF consumption, we determined the concentration of a panel of known EDCs within blood and seminal plasma of participants prior to and after each dietary intervention. As expected, the bioactive food marker of citrus fruit, N,N-dimethyl-L-proline (ProBet), was elevated only after consumption of the ultra-processed diet, as a beverage containing citrus fruit extract accompanied only that diet (Tables S3 and S4). Intake of the ultra-processed versus the unprocessed diet led to decreased lithium (Li) levels in serum, whole blood, and seminal fluid and decreased mercury (Hg) in serum and whole blood, with a concurrent trend in semen (Figure 4A; Table S4). As Li is implicated in the regulation of mood, altered Li levels may be associated with impaired mental health. Compared with the unprocessed diet, consumption of an ultra-processed diet did not change anxiety or stress levels but tended to increase the depression score in the adequate calorie arm only (Table 1). Consumption of the ultra-processed versus the unprocessed diet tended to increase

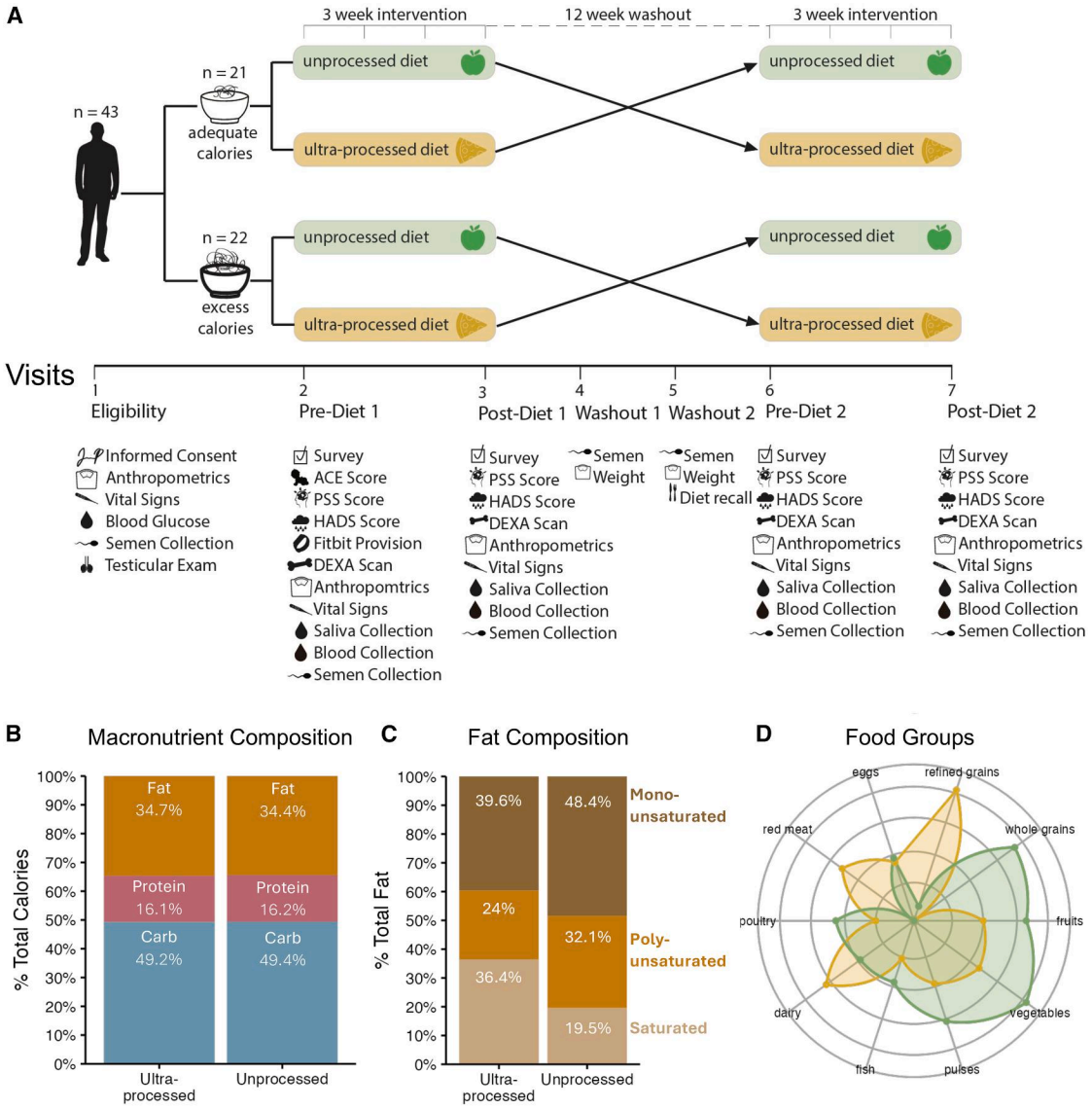

Figure 1. Characterization of the nutritional intervention

(A) Schematic of the crossover study design, identifying parameters measured at study center visits.

(B) Macronutrient contribution of the unprocessed and processed dietary regimens.

(C) Percentage of fats from each dietary regime coming from saturated, monounsaturated, or polyunsaturated fatty acids.

(D) Food group allocations in each of the dietary interventions, represented as the number of standard serving sizes. Radar chart axis is on a log scale.

the accumulation of mono(4-methyl-7-carboxyheptyl)phthalate (cxMINP) in serum and to decrease serum levels of PFAS compounds perfluoro octanesulfonic acid (PFOS) and perfluoro heptanoic acid (PFHpA) (Figure 4A; Table S4). Compared with baseline, consumption of the unprocessed diet was associated with increased Li levels in serum, whole blood, and semen (Table S3). The concentrations of Hg were also increased in all compartments after the unprocessed diet, and a trend increase was detected after the ultra-processed diet in blood and serum (Table S3).

Consumption of the unprocessed versus ultra-processed diet altered markers of systemic inflammation and oxidative stress, with increased interleukin-4 (IL-4) in the adequate calorie arm (Figure 4B; Table S5) and a trend of decreased monocyte chemoattractant protein-1 (MCP-1) levels in the excess calorie arm (Figure 4C; Table S5). C-reactive protein (CRP) and 8-oxo-7,8-dihydro-2-deoxyguanosine (8-oxo-dg), a marker of oxidative stress, were unchanged (Figures 4D and 4E; Table S5).

DISCUSSION

In this dietary intervention study, we found that UPF consumption influences cardiometabolic and reproductive health. Diets also caused a trend in the differential accumulation of contaminants in blood and seminal fluid, with Li and Hg displaying a decreased accumulation and serum cxMINP displaying a trend

Table 1. Alterations of physiological characteristics in response to unprocessed versus ultra-processed diet

	Δ Unprocesssed → ultra-processed				
	Calorie level	Baseline (mean ± SD)	Estimate	*p* adjusted	95% CI
Anthropometric characteristics					
Weight (kg)	adequate	78.94 ± 9.45	**1.4055**	**0.027**	**0.566**; **2.251**
	excess	76.57 ± 8.86	**1.2768**	**0.032**	**0.467**; **2.083**
Fat mass (kg)	adequate	14.46 ± 4.25	**0.9980**	**0.027**	**0.405**; **1.641**
	excess	15.75 ± 5.11	**0.9555**	**0.027**	**0.377**; **1.533**
Lean mass (kg)	adequate	64.05 ± 7.5	0.2204	0.829	−0.675; 1.107
	excess	60.97 ± 7.49	−0.321	0.679	−1.176; 0.533
Blood pressure					
Systolic blood pressure (mmHg)	adequate	123.07 ± 8.32	0.3231	0.907	−3.023; 3.798
	excess	125.28 ± 9.42	2.2821	0.344	−0.924; 5.486
Diastolic blood pressure (mmHg)	adequate	74.6 ± 6.38	2.5624	0.244	−0.435; 5.684
	excess	75.51 ± 6.85	**4.8286**	**0.027**	**1.917**; **7.730**
Liver function					
Alanine transaminase (U/L)	adequate	22.55 ± 13.92	12.7594	0.125	**1.609**; **23.926**
	excess	20.71 ± 9.63	−2.188	0.862	−12.84; 8.499
Aspartate aminotransferase (U/L)	adequate	21.6 ± 8.02	−0.669	0.906	−6.986; 5,648
	excess	22.33 ± 5.19	−2.434	0.669	−8.491; 3.624
Alkaline phosphatase (U/L)	adequate	61.04 ± 16.91	3.0979	0.512	−2.497; 8,693
	excess	55.83 ± 18.53	−6.603	0.0.099	**−11.97**; **−1.238**
Albumin (g/L)	adequate	33.28 ± 3.59	−0.125	0.941	−2.738; 2.488
	excess	33.54 ± 3.6	−3.209	0.089	**−5.715**; **−0.703**
Pancreatic function					
Blood glucose (mmol/L)	adequate	5.05 ± 0.35	0.0135	0.941	−0.256; 0.284
	excess	5.07 ± 0.35	−0.099	0.679	−0.358; 0.160
HbA1C (mmol/mol)	adequate	31.9 ± 2.96	0.1019	0.89	−0.550; 0.751
	excess	32.45 ± 2.4	−0.030	0.941	−0.658; 0.585
Insulin (pmol/L)	adequate	34.24 ± 17.65	14.9381	0.162	**0.090**; **29.787**
	excess	40.64 ± 30.52	−14.42	0.162	**−28.66**; **−0.184**
C-peptide (pmol/L)	adequate	402.4 ± 124.3	**124.7058**	**0.052**	**38.5**; **211.87**
	excess	443.9 ± 169.7	−42.94	0.54	−125.6; 40.236
Thyroid function					
T4 (nmol/L)	adequate	66.6 ± 11.11	0.6162	0.906	−4.720; 5.952
	excess	65.79 ± 9.12	−5.722	0.132	**−10.84**; **−0.604**
Total T3 (nmol/L)	adequate	1.4 ± 0.17	0.1268	0.099	**0.024**; **0.233**
	excess	1.5 ± 0.2	−0.045	0.601	−0.145; 0.054
TSH (miU/L)	adequate	1.78 ± 0.9	0.4949	0.089	**0.110**; **0.884**
	excess	1.96 ± 0.68	−0.207	0.5	−0.573; 0.165
Appetite hormones					
FGF-21 (pg/mL)	adequate	74.98 ± 59.92	30.0376	0.305	−9.260; 69.335
	excess	98.41 ± 88.18	−4.172	0.906	−41.67; 33.606
GDF-15 (pg/mL)	adequate	313.5 ± 71.8	−10.01	0.718	−40.65; 20.753
	excess	357.8 ± 155.2	**−51.85**	**0.027**	**−81.22**; **−22.76**
Ghrelin (pg/mL)	adequate	882.57 ± 308	66.0450	0.599	−73.35; 205.71
	excess	886.3 ± 304.2	60.4635	0.601	−74.14; 192.4
Glucagon (pmol/L)	adequate	4.01 ± 1.52	4.1106	0.144	**0.235**; **7.970**
	excess	5.21 ± 3.29	0.4144	0.906	−3.293; 4.124
Leptin	adequate	3,010 ± 2,538	1,316.78	0.176	−40.53; 2,667.9
	excess	3,738 ± 3,508	1,706.594	0.084	**417.1**; **2,994**

(*Continued on next page*)

Table 1. ***Continued***

	Δ Unprocesssed → ultra-processed				
	Calorie level	Baseline (mean ± SD)	Estimate	*p* adjusted	95% CI
Lipid panel					
Total cholesterol (mmol/L)	adequate	3.34 ± 0.76	**0.5444**	**0.027**	**0.207**; **0.879**
	excess	3.53 ± 0.68	0.2823	0.228	−0.036; 0.608
Triglyceride(mmol/L)	adequate	0.68 ± 0.25	0.0942	0.512	−0.077; 0.265
	excess	0.74 ± 0.39	0.0022	0.98	−0.161; 0.166
LDL:HDL ratio	adequate	1.85 ± 0.61	**0.3851**	**0.027**	**0.171**; **0.603**
	excess	2.06 ± 0.69	0.2454	0.11	**0.039**; **0.453**
Semen quality					
Semen volume (mL)	adequate	4.03 ± 1.66	−0.125	0.862	−0.741; 0.490
	excess	3.36 ± 0.98	0.5057	0.244	−0.085; 1.096
Sperm conc. (million cells/mL)	adequate	73.45 ± 54.33	−9,397	0.679	−34.78; 15.981
	excess	71.95 ± 42.3	−23.12	0.189	−47.46; 1.214
Sperm morphology (%)	adequate	2.81 ± 1.33	−0.168	0.889	−1.274; 0.939
	excess	3.14 ± 1.52	0.6502	0.459	−0.411; 1.712
Sperm total motility (%)	adequate	73.81 ± 19.12	−5,869	0.634	−19.48; 7.744
	excess	74.95 ± 20.07	−13.23	0.162	**−26.28**; **−0.176**
Reproductive hormones					
FSH (IU/L)	adequate	3.58 ± 1.79	−0.255	0.323	−0.598; 0.087
	excess	3.59 ± 1.24	**−0.522**	**0.027**	**−0.851**; **−0.193**
LH (IU/L)	adequate	4.94 ± 1.9	−0.3	0.751	−1.294; 0.697
	excess	4.91 ± 1.69	−0.639	0.395	−1.594; 0.317
Testosterone (nmol/L)	adequate	18.71 ± 4.49	−2.111	0.142	**−4.071**; **−0.152**
	excess	15.85 ± 4.21	−0.679	0.679	−2.558; 1.200
Mental health					
Anxiety score	adequate	4.52 ± 3.11	−1,234	0.228	−2.619; 0.197
	excess	5.86 ± 3.23	−1.075	0.1285	−2.432; 0.280
Depression score	adequate	3.05 ± 3.11	1.3106	0.134	**0.123**; **2.499**
	excess	3.86 ± 2.53	−0.205	0.879	−1.355; 0.945
Stress score	adequate	16.33 ± 8.22	0.8557	0.831	−2.738; 4.544
	excess	21.5 ± 9.05	0.6076	0.879	−2.902; 4.124

Change in physiological characteristics in response to unprocessed versus ultra-processed diet determined via mixed linear model analysis on the difference between pre- and post-diet values for the two discordant diets. The model explored the interaction of diet type and caloric arm, while controlling for diet order and paired participant data. Baseline value for each characteristic is reported as mean and standard deviation (SD) for all participants at study center visit 2 (pre-diet 1). *p* value is Benjamini-Hochberg adjusted, wherein family-wise error rate is determined across all variables within table. Estimated alteration in change between diets and 95% CI is reported for each physiological characteristic in each arm independently. Bold numbers denote statistical significance.

of increased accumulation in the ultra-processed compared with the unprocessed diet. By providing a fixed number of calories to study participants during both diets, we were able to determine that the processed nature of the food itself, independent of the caloric and macronutrient intake, impacts numerous health markers. By study design, previous work identifying that UPFs induce higher caloric intake could not determine the effect of the processed nature of the food alone.[24,25] In the present study, many clinical parameters, including fat mass, LDL:HDL ratio, and markers of reproductive health, were differentially affected by adequate or excess energy intake, indicating that both caloric intake and the processed nature of the food are likely to contribute to the deleterious effect of UPF consumption.

Despite caloric matching of discordant diets, we observed differences in body weight accumulation between diets, which appear to reflect changes in fat mass. This uncoupling between total energy consumed and body weight suggests that total caloric intake is not the sole determinant of body weight gain. Factors such as a decrease in the metabolizable energy available from unprocessed versus UPFs[27] and/or alterations in metabolic rate caused by dietary-driven hormonal changes, such as GDF-15,[28] could contribute to altered energy balance. Similarly, in a previous study, increased caloric intake driven by ultra-processed versus unprocessed diet did not fully account for the body weight increase.[24] Based on the estimated relationship between caloric intake and body weight change,[29] the total energy difference of 7,100 kcal between the ultra-processed and

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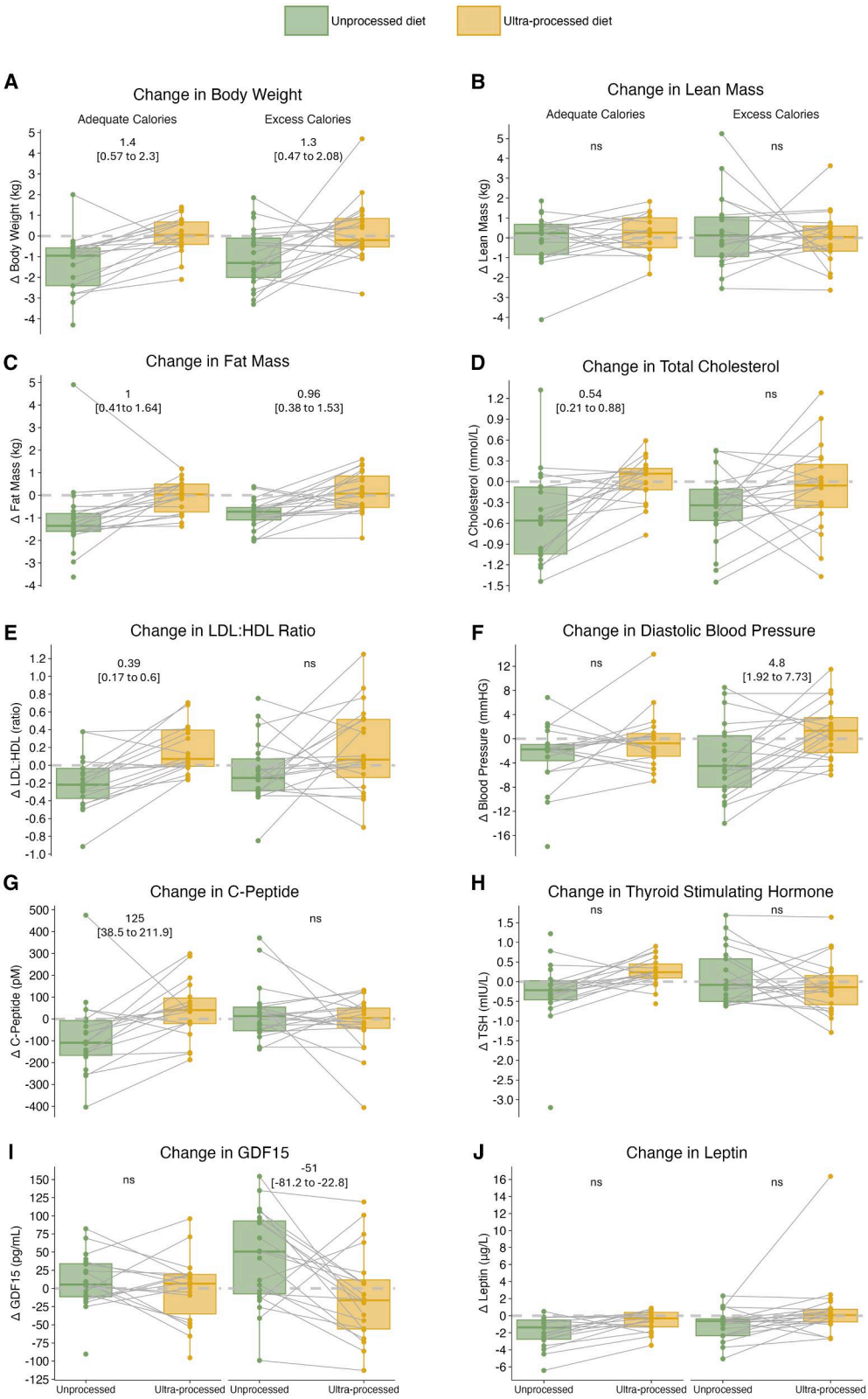

Figure 2. Alteration of metabolic characteristics following dietary intervention

(A) Alteration in body weight over dietary periods.

(B and C) Alteration in (B) lean mass and (C) fat mass over dietary periods, obtained via DEXA scan.

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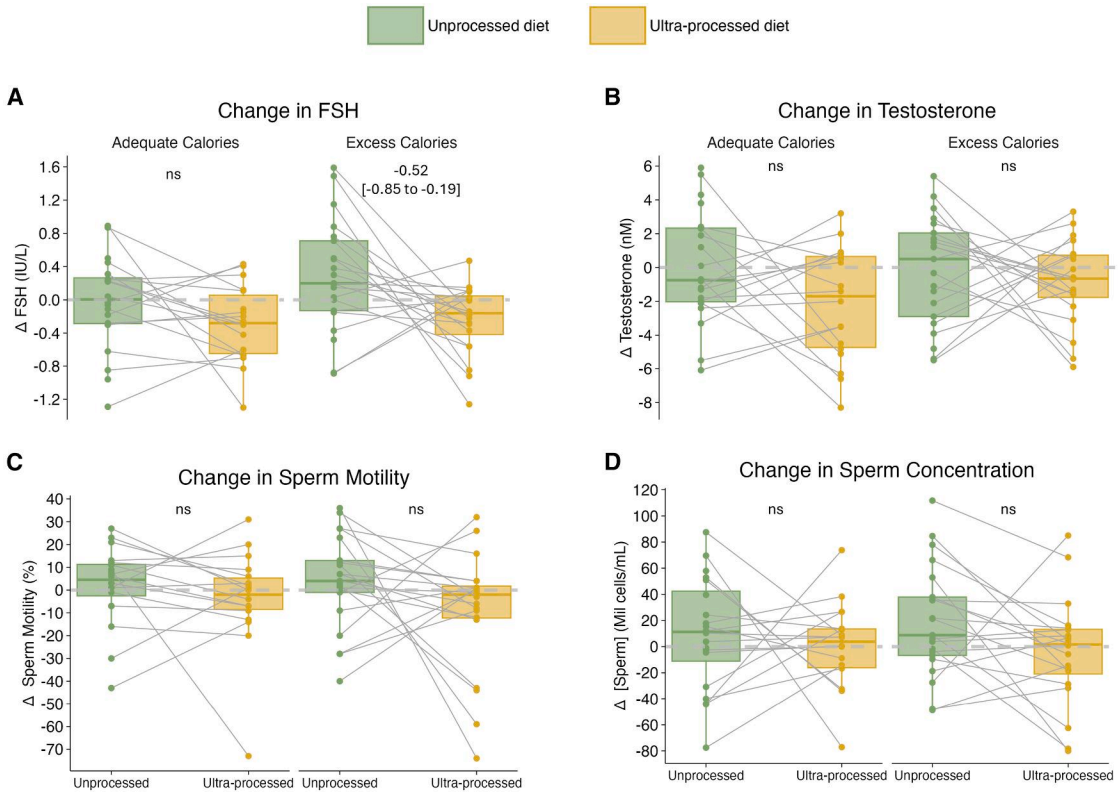

Figure 3. Alteration of reproductive characteristics following dietary intervention

(A and B) Alteration in (A) serum FSH concentration and (B) in serum testosterone concentration over the dietary periods.

(C and D) Alteration in (C) total sperm motility percentage and (D) sperm concentration over the dietary periods.

Box represents IQR and whiskers are ±1.5 × IQR. Dots indicate individual observations and paired data indicated by gray lines. Effect size and 95% CI of difference in parameter change from baseline following unprocessed versus processed diet indicated for each study arm when *p*adj < 0.05.

unprocessed diet consumption across the 2 weeks' intervention was expected to lead to approximately 1 kg of weight gain, whereas study participants exhibited a difference in body weight of 1.8 kg across the intervention.[24] Thus, the aggregation of this response to UPF in this latter study with our study provides evidence that calories from unprocessed or UPFs are not equally stored or metabolized, even when controlled for macronutrient load.

Although reproductive and cardiometabolic health appeared to improve after consumption of the unprocessed compared with the ultra-processed diet, we identified a pro-inflammatory signature in response to the unprocessed diet, with interferon (IFN)-γ and CRP increased and the anti-inflammatory cytokine IL-4 decreased, compared with baseline levels. These results, linking unprocessed-diet-induced weight loss and increased inflammation, may appear counter intuitive because diet-induced weight loss is typically associated with decreased inflammation.[30] However, most, if not all, intervention studies reporting effects of weight loss on inflammation are conducted in individuals with obesity. In our study, participants were lean; thus, weight loss in this population may trigger a different response. Increased inflammation in response to the unprocessed diet may be the consequence of an abrupt dietary shift from the habitual diet, causing an adaptive inflammatory response. Indeed, the habitual diet of participants was composed of 51% of calories from UPFs (Figures S3D and S3E), whereas the experimental unprocessed diet comprised less than 1% and the experimental ultra-processed diet 77% (Table S1). Such an adaptive response to the experimental diets may not reflect a stable response, as previously commented on.[31] Importantly, this also highlights the necessity to contrast the response to ultra-processed against unprocessed diets to isolate the effect of UPF consumption in populations already consuming substantial amounts of UPFs.

(D and E) Alteration in (D) total cholesterol and (E) LDL:HDL ratio over dietary periods.

(F) Alteration in diastolic blood pressure over dietary periods.

(G) Alteration in C-peptide over dietary periods.

(H) Alteration in TSH over dietary periods.

(I and J) Alteration in appetite hormones (I) GDF-15 and (J) leptin over dietary periods.

Box represents IQR and whiskers are ±1.5× IQR. Dots indicate individual observations and paired data indicated by gray lines. Effect size and 95% CI of difference in parameter change from baseline following unprocessed versus processed diet indicated for each study arm when *p*adj < 0.05.

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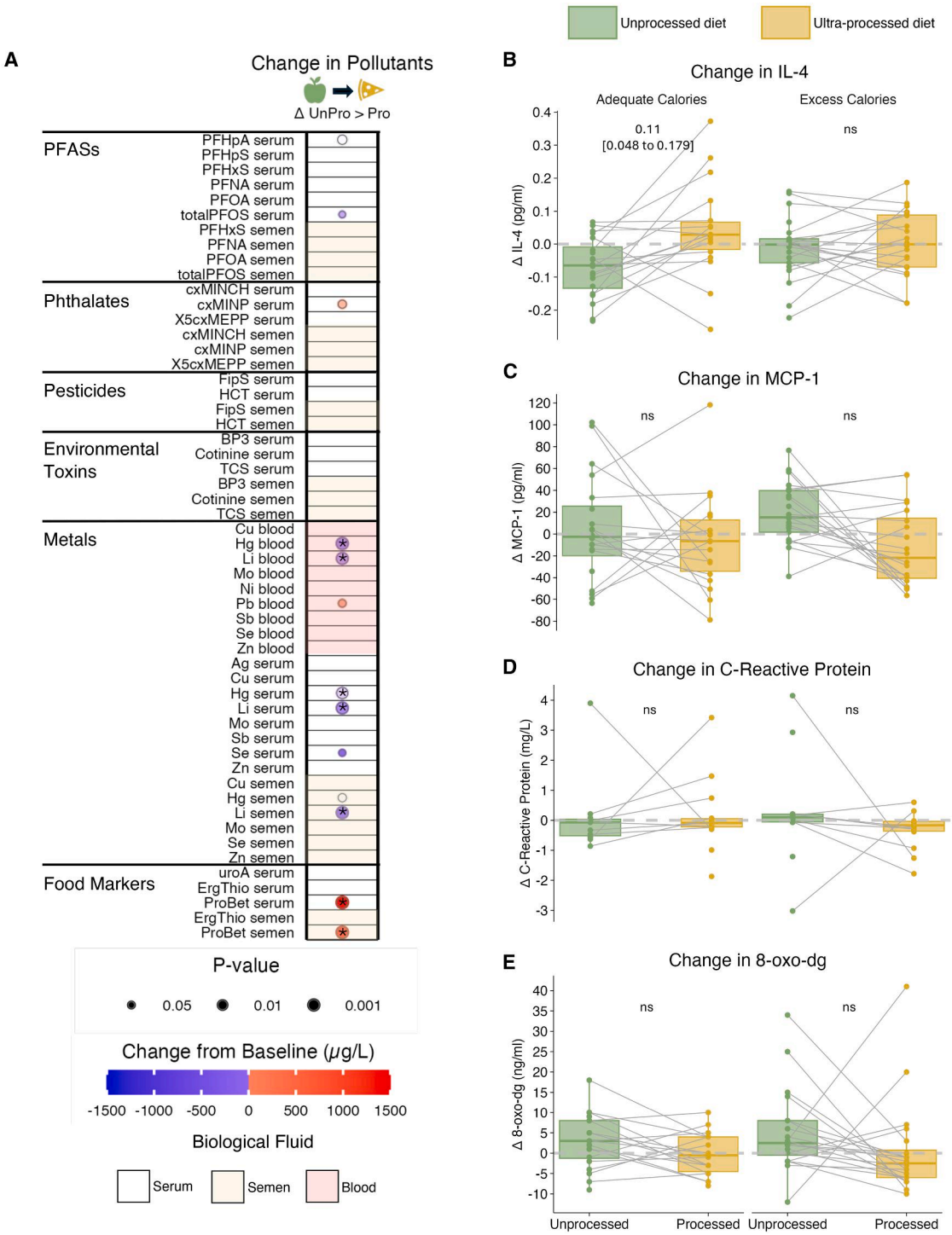

Figure 4. Alteration of pollutants and inflammatory markers following dietary interventions

(A) Alteration in concentration of a panel of pollutants, metals, and bioactive food markers between comparison of concentration changes between unprocessed versus ultra-processed diet. Non-corrected alterations of compounds indicated by dots, with size indicating significance level and color indicating direction and magnitude of alterations. Asterisk (*) indicates statistically significant ($p > 0.05$) alteration after false discovery rate (FDR) correction; FDR correction for statistical significance is considered independently as the family-wise error rate (FWER) of toxins alone. Compounds measured in serum, semen, and whole blood.
(B–D) Alteration in concentration of inflammatory markers (B) IL-4, (C) MCP-1, and (D) CRP over the dietary periods; single outlier removed from visualization of CRP.

(legend continued on next page)

Many contaminants were detected at lower levels upon completion of each of the experimental diets compared with baseline (Table S3). This suggests that our experimental diets were less contaminated with pollutants than the habitual diets of participants. Thus, our measures of pollutants may not reflect real-life exposure but rather underestimate the pollutant exposome associated with UPF consumption. Nevertheless, our *cleaner* experimental diets identified several pollutants accumulated in bodily fluids after ultra-processed versus unprocessed diet consumption, such as a trend for the phthalate compound MINP, a secondary metabolite of diisononyl phthalate (DINP). Given the documented deleterious effect of DINP on cardiovascular health,[32] the brain,[33] and reproductive function,[34,35] increased exposure to phthalates in UPF may contribute to the observed effects on mood and reproductive health changes. It is, however, unclear whether cxMINP acts as an endocrine disruptor, as serum cxMINP levels were not associated with changes in hormone levels (Figure S2). Yet serum levels of PFHpA, a compound that tended to be less present after the ultra-processed compared with the unprocessed diet, were positively associated with levels of thyroid hormone T4 (Figure S2). Although endocrine disruption due to accumulated PFHpA from UPF consumption would be in line with studies on PFHpA reporting effects on thyroid function, cholesterol levels,[36] and spermatogenesis,[37] more investigations are warranted to test such potential causal relationships.

In conclusion, our results demonstrate that consumption of UPF itself, irrespective of excess caloric intake, is detrimental to human health. Moving dietary patterns away from UPF and toward less-processed alternatives may promote cardiometabolic and mental health, along with amelioration of male reproductive fitness.

Limitations of the study

Although the free-living nature of our study has undeniable advantages compared with inpatient experiments, notably in relation to feeding behavior, it carries inherent limitations. Due to the study design, the estimation of energy intake relied on participants' adherence to the nutritional intervention and the accuracy of their reporting. Although adherence was assessed through detailed daily questionnaires and questionnaires at the end of each treatment period, we cannot rule out potential bias in the reporting of actual caloric intake. This may affect our ability to determine whether the effects of the UPF diet are dependent on caloric intake. However, the similar effects observed on body composition markers in both the adequate and excess caloric study arms suggest that the detrimental effects of the ultra-processed diet compared with the unprocessed diet are not solely attributable to caloric intake but extend across both study arms.

Another limitation of our study is the relatively short duration of the experimental diets, which may not reveal stable effects of chronic diet consumption; 3-week diet interventions may have induced acute responses that may normalize with time if diets were prolonged. Although the ultra-processed diet unlikely triggered an adaptive response due to the similar amounts of UPFs in the experimental diet compared with the habitual diets of participants, the unprocessed diet represented an important shift compared with their habitual diets and may have induced acute responses, as suggested by the increased levels of inflammatory markers after the diet period. As we did not measure inflammatory markers in a time-course fashion, we cannot determine whether elevated markers of inflammation are normalizing at the completion of the unprocessed diet, as would be expected after an acute response. Regardless of potential long-lasting effects of unprocessed food consumption, the beneficial effects of a 3-week consumption period of unprocessed foods may constitute an intervention for individuals consuming >50% of energy from UPFs and aiming to ameliorate cardiometabolic and reproductive health.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to the lead contact, Romain Barrès (barres@ipmc.cnrs.fr).

Materials availability

This study did not generate new, unique reagents.

Data and code availability

All raw data used to generate graphs are included in Data S1. The data that support the findings of this study are available from the corresponding author upon reasonable request.

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AUTHOR CONTRIBUTIONS

Conceptualization, J.M.P., R.B., S.J.S., and M.A.N.; formal analysis, S.J.S., R. B., and C.L.; methodology, J.M.P., J.T., V.G., L.Ä., S.H., J.M.C., S.T., and K.S. H.; investigation, J.M.P., J.I., A.H., L.H., J.T., C.S., A.N.H., L.Ä., and S.H.; writing – original draft, R.B.; writing – review & editing, all authors; supervision, R.B. and J.M.C.

DECLARATION OF INTERESTS

S.T. receives honoraria from Merck, Ferring, and Novo Nordisk; is part of the consulting board for Novo Nordisk; and is a recipient of research funding from Novo Nordisk.

(E) Alteration in serum 8-oxo-dg concentration over the dietary periods. FDR correction for statistical significance is considered independently as the FWER of inflammatory markers alone.

Box represents IQR and whiskers are ±1.5 × IQR. Dots indicate individual observations and paired data indicated by gray lines. Effect size and 95% CI of difference in parameter change from baseline following unprocessed versus processed diet indicated for each study arm when $p$adj < 0.05.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Deposited data		
Data S1 – Source Data	This paper	N/A
Software and algorithms		
Schofield equation	Schofield[38]	https://www.scirp.org/pdf/Health_2014052110591581.pdf
Software package: R version 4.2.1	R Core Team	https://www.r-project.org/
Other		
NOVA food processing scale	Monteiro et al.[7]	https://doi.org/10.1017/S1368980017000234

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

This study reports a subset of exploratory secondary outcomes defined in the protocol registered on ClinicalTrials.gov with identifier NCT05368194. The primary outcome was DNA methylation in sperm and is not reported here and will be reported in a subsequent publication. Outcomes reported in this study were initially selected as the most relevant clinical outcomes in each organ function category and before statistical testing was applied. The study protocol was approved by the research ethics committee of the Capital Region of Denmark (De Videnskabsetiske Komiteer for Region Hovedstaden) as protocol identifier: H-20061598. All participants consented to the study.

The following characteristics were defined as inclusion criteria, evaluated at baseline: male, 20 to 35 years old, BMI between 18.5 and 30, semen quality above the WHO lower reference, and no evidence of cardiometabolic, psychiatric, or reproductive disorder. Exclusion criteria included: history of serious or chronic illness, history of obesity, any food restrictions, current use of drugs, alcohol (>14 units per week and/or recurring binge drinking), and/or tobacco/nicotine products, use of prescription medication, and > 200 minutes of vigorous aerobic exercise per week. A CONSORT diagram showing numbers of participants in each step of the protocol is shown in Figure S4.

Nutrition Intervention

The nutritional interventions consisted of four dietary treatments supplied for 3 weeks each. The interventions included the two discordant diets at either calorically adequate or excess quantities (Figure 1A). Pre-prepared and portioned meals were provided by local food producer *Mydietpal* to the homes of participants, and left-over/additional food intake was monitored by daily questionnaires to estimate total energy intake. Pictures of typical meals are provided in Data S2. Caloric load was determined using the Schofield equation[38] based on weight, activity multiplier and age at baseline, and participants received caloric quantities based on their estimated energy expenditure (EEE) (adequate), or 500 calories/day more than their EEE (excess). Unprocessed and ultra-processed diets were matched for caloric load, macronutrient makeup and meal contribution. Food processing was determined using the NOVA food processing scale.[7] Our aim was to design an ultra-processed diet with >75% of calories derived from NOVA 4 category foods and an unprocessed diet with >75% of calories derived from NOVA 1 and NOVA 2 category foods. The exact contribution from each NOVA category were obtained after matching diets for calorie, macronutrient, and food group goals and are as follows: for the *unprocessed diet*, 66% unprocessed (NOVA 1), 6% culinary processed (NOVA 2), 28% processed (NOVA 3) and <1% from ultra-processed foods (NOVA 4), while for the ultra-processed diet, 5% NOVA 1, 6% NOVA 2, 11% NOVA 3 and 77% from NOVA 4 (Table S1). Participant compliance and deviation from the nutrition intervention were assessed via detailed daily questionnaires and validated from questionnaires at the end of each treatment period. The NOVA composition of the diet consumed in each group after correction by these questionnaires is shown in Table S6.

The study was administered as a dual arm 2x2 crossover study design (Figure 1A). Treatments were punctuated by a 12-week washout period. The study arms were (1) the adequate and (2) the excessive calorie arms. Within each arm, all participants received both the unprocessed and the ultra-processed diet, with randomization of diet order. Group randomization was performed by a blinded member of the team following eligibility assessment, wherein groups were blocked to ensure equal participants per treatment group, followed by stratification to ensure no statistically significant deviation in age and BMI at baseline per group.

Participants attended seven study center visits, including an eligibility assessment, two pre-diet visits, two post-diet visits, and two washout period visits. At each pre- and post-diet study center visit, participants underwent survey administration, anthropometric characterization, vitals measurement, dual energy x-ray absorptiometry (DXA) scanning, peripheral blood collection, and semen collection by assessors blinded to treatment group allocation.

METHOD DETAILS

Semen quality analysis

Within 90 minutes of semen collection, semen quality analysis was determined. Semen volume was estimated by sample weight. Semen pH, sperm concentration and motility were determined using the LenseHooke sperm quality analyzer (SQA) and test cassette (LenseHooke. X1 Pro and Lensehooke CS1 Semen Test Cassette). Seminal plasma was isolated by two centrifugations at 1000g, 10 minutes, room temperature and stored at -80°C.

Peripheral blood processing

Peripheral blood was collected in serum separator (BD serum), sodium heparin/Ficoll (Bd Vacutainer CPT), and EDTA tubes (BD Vacutainer EDTA). Serum from serum separator tubes and plasma from EDTA tubes were isolated via centrifugation, 3000 RPM, 4°C, 10 minutes, and stored at -80°C. Clinical biochemical parameters were assessed at the clinical biochemistry section of Rigshospitalet, Copenhagen University Hospital, Copenhagen, Denmark, using the procedures specified in the Rigshospitalets Labportal.[39] Hemoglobin A1C (HbA) status was determined on frozen EDTA whole blood at Steno Diabetes Center, Copenhagen, Herlev, Denmark. Appetite hormones and inflammatory markers were determined in frozen EDTA plasma samples using ELISA-based immune assays.

Synthetic chemical analysis

A variety of pollutants, toxicants, metals and bioactive food compounds were measured using quantitative mass spectrometry approaches at the Applied Mass Spectrometry in Environmental Medicine lab at Lund University. A panel of organic pollutants was determined in frozen serum, blood and seminal plasma. This panel included PFASs [Perfluoro heptanoic acid (PFHpA), Perfluoro hexanesulfonic acid (PDHxS), Perfluorooctanoic acid (PFOA), Perfluoro heptanesulfonic acid (PFHpS), Perfluoro nonanoic acid (PFNA), Perfluoro octanesulfonic acid (PFOS)], phthalates [mono(2-ethyl-5-carboxypentyl) phthalate (5cxMEPP), mono(4-methyl-7-carboxyheptyl)phthalate (cxMINP), 1,2- Cyclohexanedicarboxylic Acid Mono 4-Methyl-7-carboxy-heptyl Ester (cxMINCH)], pesticides [4 hydroxy chlorothalonil (HCT) and fipronil-sulphone (FipS)], environmental contaminants [Cotinine (COT), Oxybenzone-Bensophenone-3 (BP3), and Triclosan (TCS)], bioactive food compounds [Ergothioneine (Ergo) and proline betaine (ProBet)]. In addition, levels of the metals lithium (Li), nickel (Ni), copper (Cu), zinc (Zn), selenium (Se), molybdenum (Mo), silver (Ag), antimony (Sb), mercury (Hg), and lead (Pb) were determined in serum, whole blood and seminal plasma. Oxidative stress was determined based on quantification of 8-Oxo-7,8-dihydro-2-deoxyguanosine (8-oxo-dg). The laboratory participates bi-annually in the German External Quality Assessment Scheme (G-EQUAS).

QUANTIFICATION AND STATISTICAL ANALYSIS

We estimated the required number of participants based on results from previous studies with dietary interventions similar to the unprocessed and ultra-processed diets.[24,40–42] We used cholesterol as a parameter to determine a sample size with power to detect significant alteration in a physiologically metabolically relevant parameter with established dietary alteration. Sample size calculations used a power of 0.8, an expected drop-out rate of 15%, and a level of significance of 0.05, indicating that 18 men per study group were required. Therefore, we recruited a total of 43 men, resulting in 21–22 men per group who were subjected to the first and second dietary intervention.

Statistical analysis was performed using R Studio (R version 4.2.1 (2022-06-23)). For all biological variables, differences between dietary groups and interventions were determined using mixed linear model analysis on the difference between pre- and post-diet values. Models explored the interaction of diet type and arm, while controlling for diet order, utilizing a random effect of participant to account for paired data. Evaluation of contaminants used the same model without testing for interacting effect of adequate or excess calorie arm. We report effect size, 95% confidence intervals (CI) and Benjamini-Hochberg adjusted *P*-values. Family-wise error rate (FWER) was detected for all variables specified in Table 1, and independent FWER was determined independently on exploratory outcomes of inflammatory markers, pollutant accumulation, and parameter alteration from baseline.