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# A high-fat diet rich in corn oil reduces spontaneous locomotor activity and induces insulin resistance in mice

Chi Kin Wong[b,1], Amy Botta[a,1], Jason Pither[a], Chuanbin Dai[a], William T. Gibson[b], Sanjoy Ghosh[a,*]

[a]Department of Biology, IKBSAS, University of British Columbia-Okanagan, Canada
[b]Department of Medical Genetics, University of British Columbia, University of British Columbia, Vancouver, Canada

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**Abstract**

Over the last few decades, polyunsaturated fatty acid (PUFA), especially n-6 PUFA, and monounsaturated fatty acid content in 'Western diets' has increased manyfold. Such a dietary shift also parallels rising sedentary behavior and diabetes in the Western world. We queried if a shift in dietary fats could be linked to physical inactivity and insulin insensitivity in mice. Eight-week old female C57/Bl6 mice were fed either high-fat (HF) diets [40% energy corn oil (CO) or isocaloric olive oil (OO) diets] or chow ($n$=10/group) for 6 weeks, followed by estimation of spontaneous locomotor activity, body composition and *in vivo* metabolic outcomes. Although lean mass and resting energy expenditure stayed similar in both OO- and CO-fed mice, only CO-fed mice demonstrated reduced spontaneous locomotor activity. Such depressed activity in CO-fed mice was accompanied by a lower respiratory ratio, hyperinsulinemia and impaired glucose disposal following intraperitoneal glucose tolerance and insulin tolerance tests compared to OO-fed mice. Unlike the liver, where both HF diets increased expression of fat oxidation genes like PPARs, the skeletal muscle of CO-fed mice failed to up-regulate such genes, thereby supporting the metabolic insufficiencies observed in these mice. In summary, this study demonstrates a specific contribution of n-6 PUFA-rich oils like CO to the loss of spontaneous physical activity and insulin sensitivity in mice. If these data hold true for humans, this study could provide a novel link between recent increases in dietary n-6 PUFA to sedentary behavior and the development of insulin resistance in the Western world.


*Keyword:* n-6 PUFA; MUFA; Corn oil; Polyunsaturated fatty acids; Insulin resistance; Diabetes; Locomotor activity; Exercise

## 1. Introduction

Can the quality of our diets affect our physical activity levels? The relationship between activity, metabolism and diet composition has been simply believed to be a function of total caloric intake or broad macronutrient classes. Studies in foraging animals during cold winters indicate that dietary deprivation of any one or more macronutrients like proteins, fats or carbohydrates affects their behavior and spontaneous activity levels [1]. In omnivores such as rodents, changes in total macronutrient levels of the diets affect spontaneous activity [2]. It still remains unclear if the chemical composition of a macronutrient, independent of caloric intakes, can alter spontaneous activity of any animal. This question becomes crucial if we consider the recent dietary patterns and increasing physical inactivity of another omnivore, humans across the Western world, especially during childhood. Both in the United States and Canada, the number of physically active children has decreased over the last several decades [3,4], which is temporally associated with a radical shift in the chemical composition of dietary fats during this time.

As an omnivore, humans have evolved as hunter-gatherers, having consumed predominantly animal-derived proteins and fats throughout their existence [5]. However, in recent years, to protect against chronic diseases, saturated fatty acids have been extensively substituted with unsaturated fats like monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA) in Western food supply. For example, in Canada, increases in dietary fat content over the last century are attributable almost entirely to increases in MUFA and PUFA, whereas saturated fat has remained constant (Table 1) [6]. Although MUFA like palmitoleic acid, as a part of animal tissues, were abundant in traditional human diet [7], the presence of cropseed-based PUFA, especially n-6 PUFA like linoleic acid, was considerably lower and represents a relatively new addition to our dietary niche in abundance [8]. Recent data are unequivocal about the fact that, overall, North American children and youth spend between 40% and 60% of their waking hours in sedentary activities like TV watching, video games etc. [4,9]. In parallel, children in the United States and Canada consume an average of 10.16 g/day and 7.7 g/day n-6 PUFA currently [10,11]. Whether trends in physical inactivity are biologically related to current high trends of unsaturated fats is plausible but remains unknown.

The relationship between macronutrients and physical activity in humans is confounded by multiple confounders like the extent of

* Corresponding author. Department of Biology, IK Barber School of Arts and Sciences, University of British Columbia-Okanagan, ASC 369, 3333 University Way, Kelowna, BC, Canada V1V 1V7. Tel.: +1 250 807 8162; fax: +1 250 807 8001.
*E-mail address:* sanjoy.ghosh@ubc.ca (S. Ghosh).
[1] denotes equal contribution.

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Table 1
Trends in daily dietary fatty acid intakes in Canada between 1976 and 2005.

| | 1976 | 1986 | 1996 | 2005 | % Increase |
|---|---|---|---|---|---|
| Total fats (g)) | 86.49 | 88.31 | 97.98 | 102.49 | 18% |
| Total PUFA (g) | 12.56 | 14.08 | 17.78 | 19.61 | 54% |
| Total saturates (g) | 28.07 | 27.37 | 26.60 | 27.48 | 0% |
| Total MUFA (g) | 39.61 | 40.70 | 47.31 | 48.75 | 22% |

Data from Statistics Canada — Catalogue No. 21.

surrounding urbanization [12] or socioeconomic factors like income [13], which may override the desire to remain physically active. Such relationships may also be confounded by climate-related variables like sunlight hours and ambient temperature. Moreover, any result obtained from any one country or region may be too narrow in scope to elucidate such relationships. As an example, in North America, where the addition of n-6 PUFA in the food supply is widespread (currently around 7.0±%7.3% energy [14]), the lack of a control population consuming reduced PUFA precludes the use of epidemiological data to estimate a specific effect of PUFA on physical activity at the population level. As an alternative, experimental animal studies could allow us to test the direct biological effect of dietary fat composition on physical activity.

We show that a corn oil diet rich in n-6 PUFA results in loss of spontaneous activity with negative effects on whole-body metabolism, and metabolic gene expressions, leading to insulin resistance in young female mice. If these results hold true for humans, particular attention must be paid to dietary n-6 PUFA as a potential confounder when planning lifestyle interventions for the treatment/prevention of obesity and diabetes.

Table 2
Detailed composition of experimental diets.

| Ingredients (g/kg) | Olive oil diet | Corn oil diet |
|---|---|---|
| Casein | 240 | 240 |
| DL-Methionine | 3.6 | 3.6 |
| Corn starch | 150 | 150 |
| Sucrose | 298.8 | 298.8 |
| Cellulose | 50 | 50 |
| Calcium carbonate | 3.6 | 3.6 |
| Mineral Mix[a] | 42 | 42 |
| Vitamin Mix[b] | 12 | 12 |
| | *Oils* | |
| Soybean oil | 10 | 10 |
| Corn oil | 0 | 190 |
| Olive oil | 190 | 0 |
| Total | 1000 | 1000 |
| Macronutrients | % w/w | % Energy |
| High-fat diets | | |
| Protein | 21.2 | 19 |
| Carbohydrate | 44.4 | 39 |
| Fat | 20.0 | 40 |
| Total | | 4.53 kcal/g diet |
| **Normal chow** | | |
| Protein | 22.6 | 26.4 |
| Carbohydrate | 51.2 | 60.1 |
| Fat | 5.2 | 13.7 |
| Total | | 3.41 kcal/g diet |

Note: Normal chow ingredients are variable as with any semipurified diet and have not been listed below.
[a]Mineral mix (mg/g): dicalcium phosphate 500, magnesium oxide 24; potassium citrate 220, potassium sulfate 52; sodium chloride 74, chromium $KSO_4$ $12H_20$ 0.55; cupric carbonate 0.3, potassium iodate 0.01; ferric citrate 6, manganous carbonate 3.5, sodium selenite 0.01, zinc carbonate 1.6; sucrose 118.03.
[b]Vitamin mix (mg/g): vitamin A 0.8; vitamin $D_3$ 1; vitamin E 10; menadione sodium bisulfite 0.08; nicotinic acid 3; calcium pantothenate 1.6; pyridoxine HCl 0.7; riboflavin 0.6; thiamin 0.6; sucrose 978.42.

Table 3
Detailed fatty acid analysis of high-fat diets.

| Fatty acid | g/100 g of diet | g/100 g of diet |
|---|---|---|
| Σ SFA | 2.47 | 2.81 |
| Σ MUFA | 4.9 | 12.7 |
| Σ PUFA | 9.05 | 1.71 |
| Σ trans FA | 0.098 | 0.04 |
| Total fat | 17.4 | 18.2 |
| Major FA | % in CO diet | % in OO diet |
| SCFA [C6:0 to C12:0] | <0.1 | <0.1 |
| Myristic [C14:0] | 0.11 | 0.17 |
| Palmitic [C16:0] | 11.7 | 11.7 |
| Palmitoleic [C16:1n-7] | 0.11 | 0.73 |
| Stearic [C18:0] | 2.01 | 3.69 |
| Oleic [C18:1n-9] | 28.3 | 70.4 |
| Linoleic [C18:2n-6] | 58.3 | 8.16 |
| Alpha linolenic [C18:3n-3] | 1.24 | 0.98 |
| Behenic [C22:0] | 0.14 | 0.19 |
| Lignoceric [C24:0] | 0.16 | <0.1 |
| Arachidonic [C20:4n-6] | <0.1 | 0.61 |
| Eicosapentaenoic [C20:5n-3] | <0.1 | <0.1 |
| Docosapentaenoic [C22:5n-6] | <0.1 | <0.1 |
| Docosahexaenoic [C22:6n-3] | <0.1 | <0.1 |

CO, corn oil diet; OO, olive oil diet.

## 2. Experimental methods

### 2.1. Experimental animals and diet

All animal protocols were approved by the UBC's Animal Care Committee. Eight-week-old female C57/Bl6 mice were fed a high-fat (HF, 40% energy from fat, $n=10$ per HF group) or a "normal" chow (14% energy from fat, $n=10$, Lab Diet-5P76) diet for 6 weeks. The high-fat diets were isoenergetic and isonitrogenous and were prepared commercially (Harlan Teklad, Table 2). The oils used were either 19% w/w corn oil (high-n-6 group, TD.120022) or 19% w/w olive oil (high-MUFA group, TD.130128), both supplemented with 1% w/w soybean oil to avoid essential fatty acid deficiencies. Carbohydrate and protein contents for both HF diets were 44.7% and 21.2% w/w, respectively [15].

### 2.2. Fatty acid analysis of diets

Fatty acid composition of the diets were analyzed using gas chromatography (GC) by NP Analytical Laboratories (St. Louis, MO, USA) on behalf of Harlan Teklad. In brief, mice food pellets were

Table 4
Primer sequences used for quantification of mRNA levels by real-time PCR in liver and muscle.

| Gene | Primer sequences (5'-3') | GenBank Reference # |
|---|---|---|
| Peroxisome proliferator activated receptor alpha (*Ppara*) | F: AGCCTCAGCCAAGTTGAAGT<br>R: AGAGGACAGATGGGGCTCTC | NM_001113418.1 |
| Peroxisome proliferator activated receptor delta (*Ppard*) | F: ACCTGGGGATTAATGGGAAA<br>R: CCGTGGGTTTGTCTTCATCT | NM_011145.3 |
| Peroxisome proliferator activated receptor gamma (*Pparg*) | F: TGGGTGAAACTCTGGGAGATTC<br>R: GAGAGGTCCACAGAGCTGATTCC | NM_011146.3 |
| Sterol regulatory element binding transcription factor 1 (*Srebf1*) | F: CTGGAGACATCGCAAACAAGC<br>R: ATGGTAGACAACAGCCGCATC | NM_011480.3 |
| Peroxisome proliferative activated receptor gamma coactivator 1 alpha (*Pgc1a*) | F: TTGCCCAGATCTTCCTGAAC<br>R: TCTGTGAGAACCGCTAGCAA | NM_008904.2 |
| 18s ribosomal RNA (*18S rRNA*) | F: CGGCTACCACATCCAAGGAA<br>R: GCTGGAATTACCGCGGCT | NR_003278 |

Primers used to determine gene expression, both the forward primer (F) and reverse (R) primers are indicated.

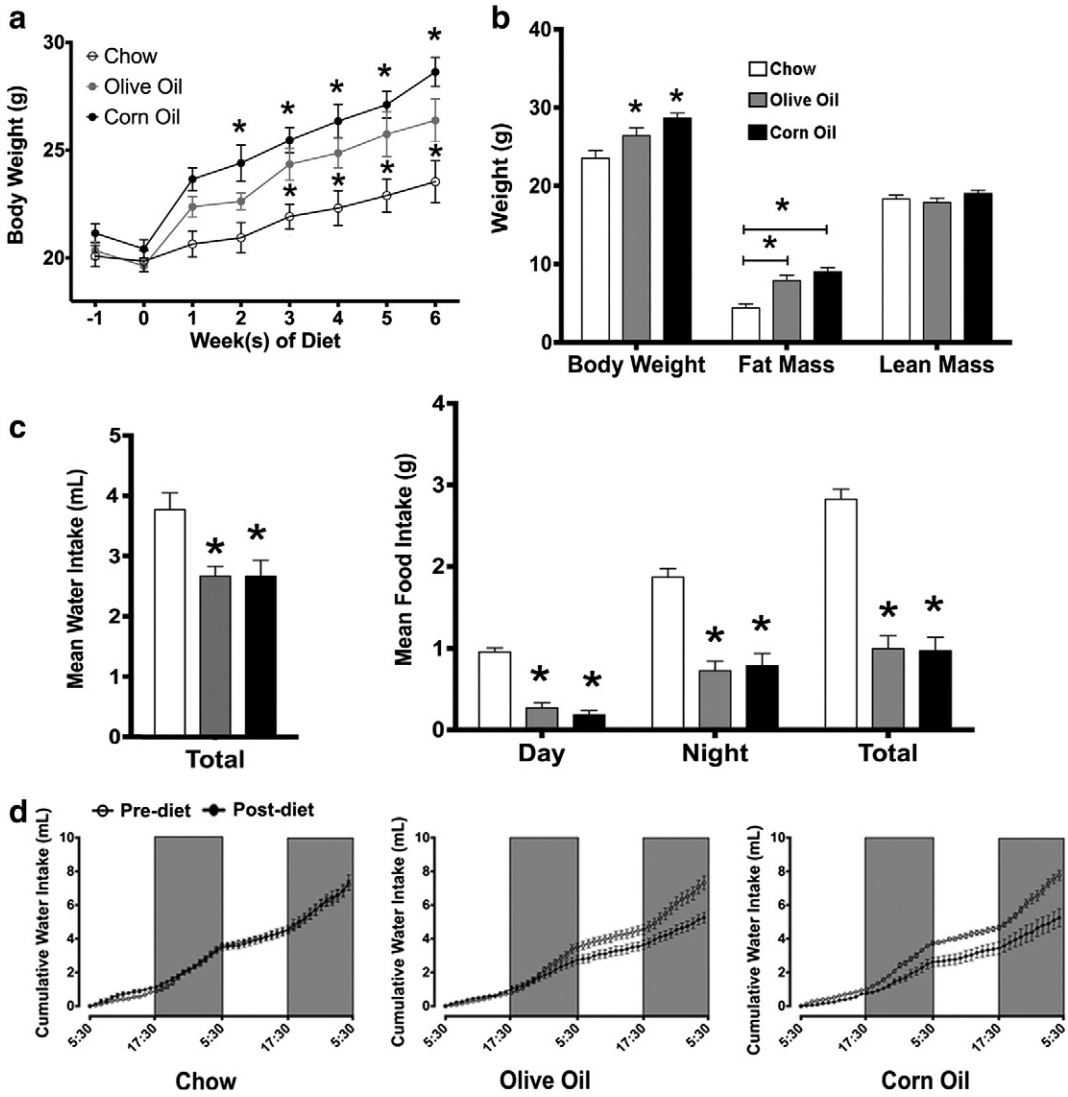


Fig. 1. Corn and olive oil feeding leads to identical gains in body weight in mice. (a) Weekly gains in body weight during dietary regimen. (b) Final body weight, fat mass and lean mass of animals after 6 weeks of diet.(c) Mean water intake of mice during each day in metabolic chamber. (d) Mean food intake of mice during each day in metabolic chambers. (e) Continious monitoring of water in mice over 2 days at night (shaded) and day in metabolic chambers. Data are presented as mean±S.E.M. ($n$=10/group) and were analyzed using one-way ANOVA with Tukey tests. *$P$<.05 versus chow.

crushed and extracted using an appropriate technique. Extracted fat was saponified with sodium hydroxide, and methyl esters of the fatty acids were formed by reaction with boron trifluoride/methanol mixture. Fatty acid methyl esters were separated by GC with a flame ionization detector. GC peak area percent of each fatty acid methyl ester was calculated as a percent of the total area of all fatty acid methyl esters.

### 2.3. Insulin and glucose tolerance tests

After 6 weeks of diet, for intraperitoneal glucose tolerance (IPGTT) testing, the animals were fasted for 5 h and blood glucose was measured (time 0). Mice were then injected intraperitoneally with 1 g/kg glucose, followed by blood glucose measurements at 15, 30, 60 and 120 min. For insulin tolerance testing (ITT), mice were fasted for 5 h, followed by basal blood glucose (time 0) measurement. Insulin was then injected intraperitoneally at 0.75 U/kg body weight. To determine glucose clearance, blood glucose was measured at 5, 15, 30 and 60 min. Over the next few days, mice were fasted and anethetized with isoflurane, their blood was collected, and they were euthanized. Kits were used to measure insulin (Linco, USA), nonesterified fatty acids (NEFA) and triglycerides (Wako, USA) in fasted serum.

### 2.4. Measurement of spontaneous activity

Following dietary protocols for 6 weeks, all mice were singly housed in metabolic cages designed to mimic home-cage environment (LabMaster TSE Systems, Bad Homberg, Germany) for 3 consecutive days. All calculated values for locomotor activity are based on measurements obtained over 48 h in the postacclimatization period. Infrared beams were oriented along the $x$, $y$ and $z$ axes of the metabolic cages which allowed for precise recording of activity levels. Spontaneous locomotor activity was analyzed by automatic recording of infrared beam breakage by animals traveling within their cages [16].

### 2.5. Measurement of metabolic parameters

In parallel to measurements of spontaneous activity, $O_2$ consumption and $CO_2$ production were measured via an open circuit indirect

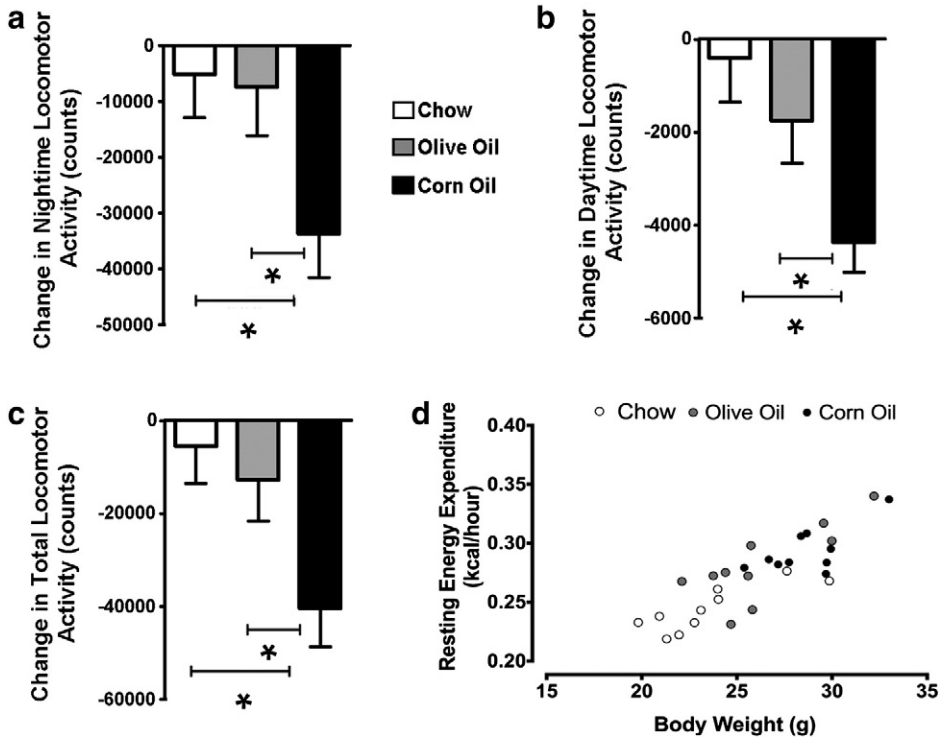


Fig. 2. Corn oil lowers spontaneous activity in mice. (a) Change in nighttime (dark phase) spontaneous locomotor activity, (b) change in daytime (light phase) locomotor activity and (c) change in total locomotor activity following 6 weeks of chow, olive-oil- and corn-oil-rich diets compared to prediet values. (d) Average REE normalized to body weight recorded from each mouse in the light phase of the day over 2 days. Data are presented as mean±S.E.M. Values (a–c) were analyzed using one-way ANOVA with Tukey tests ($n$=10/group). (d) Analyzed using ANCOVA with Tukey test; $P$<.05 ($n$=10/group). *$P$<.05 versus groups as indicated.

calorimetry system, with sensors sampling air from each cage once every 15 min (LabMaster TSE Systems, Bad Homberg, Germany) for 3 consecutive days to obtain individual measurements of respiratory function and food and water intakes [16]. Resting energy expenditure (REE) was calculated from the average of the five lowest energy expenditure recordings for each animal during the light (inactive) phase. Metabolic activity (respiratory exchange ratio, RER) was calculated from the ratio of $VCO_2$ (ml/h) produced to $VO_2$ (ml/h) consumed throughout the day for each mouse. Water intakes were continuously monitored through weight sensors directly associated with water bottles. Mice were allowed at least one dark phase period to acclimatize to the metabolic cage conditions. All calculated values for $O_2$, $CO_2$, REE and food intake are based on measurements obtained over 48 h in the postacclimatization period.

### 2.6. Body composition analysis

Animal body composition was measured in conscious animals using quantitative magnetic resonance (QMR) technology, which distinguishes differential proton states between lipids, lean tissues and free water (EchoMRI-100 Echo Medical Systems, Houston, TX, USA) [16]. Lean mass and fat mass were quantified by the QMR system.

### 2.7. Blood collection and serum parameters

Following the metabolic cage measurements, mice were fasted overnight and anethetized with isoflurane, following which animals were sacrificed, blood was collected, and plasma was separated and stored at −80°. Commercially available kits were used to measure insulin (Linco, USA), NEFA and triglycerides (Wako, USA) in these samples.

### 2.8. Gene expression analysis

mRNA levels of FA oxidation genes were quantified using qunatitative polymerase chain reaction (qPCR) from flash-frozen gastrocnemius muscle as described recently [17]. Transcription factors that regulate fat metabolism in humans and mice include peroxisome proliferator-activated receptors alpha (PPARα), delta (PPARδ) and gamma (PPARγ), as well as sterol regulatory element-binding protein 1 (SREBP-1)[18,19]. Although not an FA oxidation gene *per se*, PPAR gamma coactivator 1 (PGC-1) is capable of activating PPARα to control expression of genes encoding FA oxidation enzymes [20]. Primer sequences of analyzed genes are depicted in Table 4.

### 2.9. Statistical analysis of experimental data

Results are expressed as mean±standard error of the mean (S.E.M.). Differences between diet groups were evaluated through one-way analysis of variance (ANOVA) followed by Tukey *post hoc* tests with the exception of REE. Analysis of covariance (ANCOVA) with Tukey *post hoc* tests was used to compare REE between diet groups. For all comparisons, we used an alpha level of 0.05.

## 3. Results

### 3.1. Corn oil does not increase food intake and body weight compared to olive-oil-fed mice

The fat composition of olive and corn oil diets were similar in total saturated fatty acid (SFA; 2.47 vs. 2.81 g/100 g of diet) and n-3 PUFA (0.2 vs. 0.25 g/100 g of diet) contents, while trans fats were negligible (below 0.01% of all fatty acids) (Table 3). As expected, olive and corn oil diets had a much higher oleic acid (OA, C18:1, a MUFA) and linoleic acid (LA,

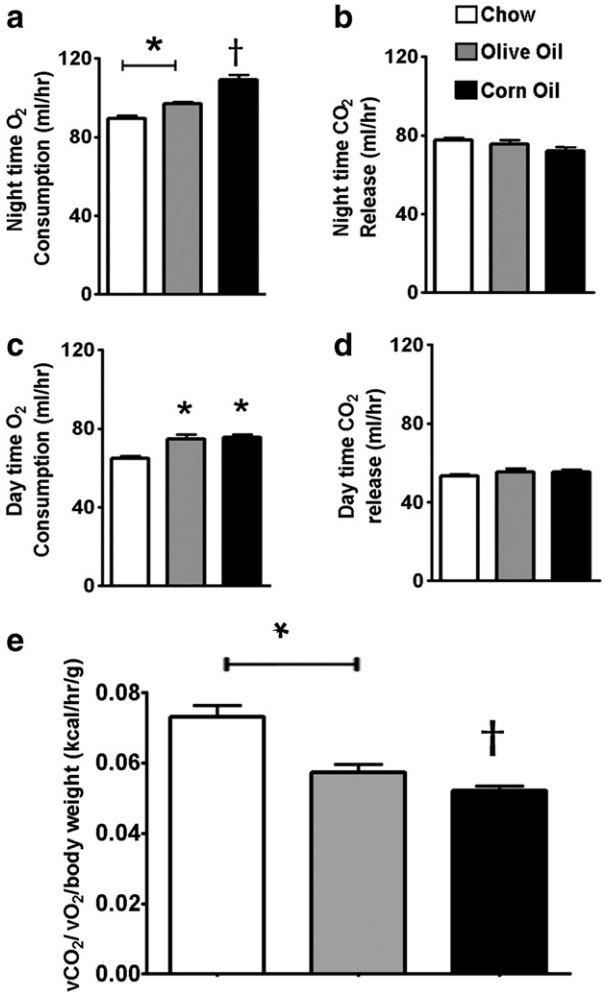


Fig. 3. Corn oil reduces whole-body RER compared to olive-oil-fed mice. RER as determined by (a) hourly nighttime (dark phase) $O_2$ intake, (b) hourly nighttime (dark phase) $CO_2$ release, (c) hourly daytime (light phase) day $O_2$ intake and (d) hourly daytime (light phase) $CO_2$ release for mice fed various diets. (e) Respiratory ratio (total $vCO_2$ released to $vO_2$ consumed per gram body weight per day) of mice fed various diets. Data are presented as mean±S.E.M. Values were analyzed using one-way ANOVA with Tukey tests ($n$=10/group). *$P$<.05 versus groups as indicated. †$P$<.05 versus all groups.

C18:2n-6, an n-6 PUFA) content, respectively (Table 3). Body weight was significantly increased in both HF-fed groups within 3 weeks of feeding, but there was no difference between olive- and corn-oil-fed mice in their final body weights after 6 weeks of HF feeding (Fig. 1a). Magnetic-resonance-imaging-based body composition analysis also demonstrated an increase in fat mass in both olive- and corn-oil-fed mice compared to chow-fed mice but no change in lean mass across all groups (Fig. 1b). With a higher energy density, mice consumed fewer grams of food and water compared to chow diets, but there was no difference in food or water intake between HF-fed groups (Fig. 1c). As dehydrated mice demonstrate increased spontaneous activity which increases their chances of locating a water source [21], continuous monitoring of water intakes also did not reveal any difference in water intake patterns between both groups of HF-fed mice at any time point during the day in both the light (day) or dark (night) phases (Fig. 1d).

### 3.2. Corn oil feeding lowers spontaneous locomotor activity in mice

Next, we used metabolic chambers to monitor spontaneous locomotor activity. Compared to chow-fed mice, PUFA-enriched corn-oil-fed mice demonstrated a threefold reduction in nighttime spontaneous activity (Fig. 2a). However, MUFA-enriched olive-oil-fed mice, despite gaining similar body weight as that of corn-oil-fed mice, maintained activity levels similar to lean, chow-fed mice (Fig. 2a). As nocturnal animals, overall activity was approximately 10-fold lower in the light phase for all mice (Fig. 2b). This difference in spontaneous activity in corn-oil-fed fed mice compared to olive-oil-fed mice continued during the daytime as well (Fig. 2b). As a result, cumulative daily activity levels were significantly lower in mice fed corn oil compared to prediet values but not in mice fed olive oil (Fig. 2c). Although spontaneous activity was different, REE was similar between chow and both groups of HF-fed mice, indicating no difference in basal energy expenditure during inactive phases in the daytime (Fig. 2d).

### 3.3. Corn oil reduces whole-body RER compared to olive-oil-fed mice

Lacking anaerobic pathways of FA oxidation, rodents consume more oxygen to oxidize fats when on HF diet [16]. Predictably, nighttime $O_2$ consumption, being at their most active time, was higher in both HF diet groups, with corn-oil-fed mice demonstrating the highest values (Fig. 3a). However, $O_2$ consumption in corn-oil-fed mice was not matched with an increased $CO_2$ release (Fig. 3b). Daytime $O_2$ consumption (Fig. 3c) and $CO_2$ release (Fig. 3d) remained unchanged among all HF groups. Overall, corn-oil-fed mice demonstrated less $CO_2$ release relative to $O_2$ consumption (denoted as RER) (Fig. 3e), indicating a lower whole-body fat oxidation in comparison to olive-oil-fed mice.

### 3.4. Corn oil feeding induces hyperinsulinemia and reduces glucose disposal in mice

A depressed RER is a characteristic feature of insulin resistance in both rodents [22] and humans [23]. To evaluate this possibility, we analyzed serum biomarkers of insulin resistance from fasted mice. Serum FA and triglycerides were unaltered, but circulating insulin increased in corn-oil-fed mice, indicating hyperinsulinemia in these mice (Fig. 4a). Corn-oil-fed mice also demonstrated insulin resistance as determined by ITT (Fig. 4b), IPGTTs (Fig. 4c) and the area under curve (AUC) of glucose disposal following ITT and IPGTT (Fig. 4d).

### 3.5. Corn oil lowers expression of multiple fatty acid oxidation genes in the muscle

To address mechanisms behind decreased RER and increased insulin resistance in corn-oil-fed mice, we analyzed gene expression of key transcription factors involved in fat oxidation in the two metabolic organs important for maintaining insulin sensitivity: the liver and the skeletal muscle. Both HF diets induced increases in the expression of PPARα, PPARγ and PGC-1α in mice livers (Fig. 5a). In contrast, PPARδ remained unchanged in the corn-oil-fed mice livers compared to chow-fed animals (Fig. 5a). Unlike in the liver, expression of all PPARs was reduced in the gastrocnemius muscle of mice fed PUFA-enriched corn oil compared to olive oil (Fig. 5b). PGC-1α was the only transcription factor that remained elevated in the corn-oil-fed mice muscle compared to olive-oil-fed mice (Fig. 5b). Like the liver, SREBP1 expression also remained unchanged in muscle in response to HF diets (Fig. 5b).

Overall, corn-oil-fed mice demonstrated lower spontaneous activity, higher insulin insensitivity, and reduced RER and skeletal muscle fatty acid oxidation gene expressions compared to similarly weighed olive-oil-fed mice.

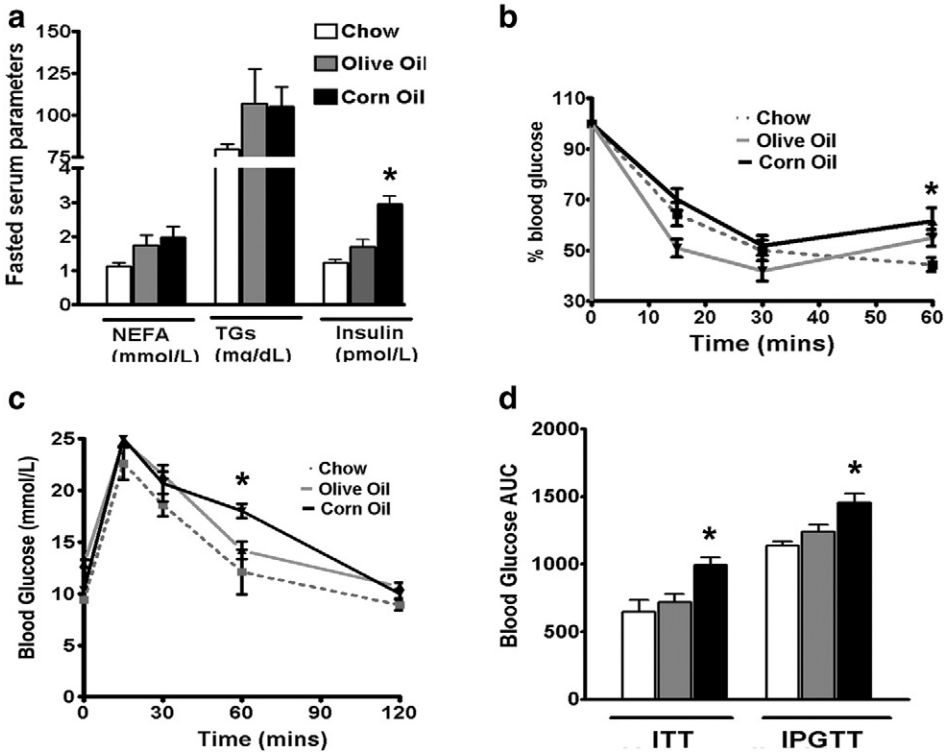


Fig. 4. Corn oil induces insulin resistance in mice. (a) Fasting serum NEFA, triglycerides and insulin; (b) ITT with blood glucose values expressed as a percentage of time 0, (c) IPGTT, (d) AUC of blood glucose values from ITT and IPGTT in mice. Data are presented as mean±S.E.M. Values were analyzed using one-way ANOVA with Tukey tests; $P<.05$ ($n=6$). *$P<.05$ versus chow-fed mice.

## 4. Discussion

Over the last 40 years, a synergistic effect of improper diet and lack of physical activity is blamed for the rapid rise in various chronic diseases like obesity, diabetes and cancer. Several studies have investigated associations between socioeconomic and environmental factors and physical inactivity in humans [24]. However, a biological link between the chemical composition of dietary macronutrients and physical inactivity has not been reported. As the chemical composition of dietary proteins and carbohydrates has remained relatively constant, the presence of n-6 PUFA in the human dietary environment is a relatively recent change, which warrants further study. Although there is considerable evidence in the literature on multiple detrimental effects of saturated fat, the metabolic effects of high dietary n-6 PUFA remain scarce. This is primarily due to the fact that popular commercial HF diets for rodent research are still mostly composed of saturated fat sources like lard and beef tallow which increase shelf life, palatibility and the ease of 'pelleting' of such diets. Unfortunately, these fat sources are not significant sources of n-6 PUFA, which contribute a large percentage of dietary energy in 'Western' diets. To the best of our knowledge, this report for the first time demonstrates a direct relationship between n-6 PUFA intake and a loss of spontaneous locomotor activity in mice in a metabolic cage, removed from any human interference for over 3 days post dietary intervention and associated insulin resistance.

In recent years, there has been a paradigm shift in our understanding of the role of lipids in obesity and cardiometabolic diseases. Several recent meta-analysis groups have questioned the role of saturated fats in causing diabetes and cardiometabolic diseases [25,26]. It has been argued that 'replacement nutrients' substituted for saturated fats in Western diets are to blame for the recent rises in obesity and associated metabolic disorders. Although simple carbohydrates are now universally condemned for their obesogenic and detrimental cardiovascular effects, reports have also linked such effects to be due to a preponderance of n-6 PUFA in the 'Western diet' [27–29]. In this regard, the proinflammatory effect of n-6 PUFA had been identified long back *in vitro* in isolated human cells and rodents [30–33].

With regards to inflammation, a clear sex difference exists with the metabolic effects of PUFA *in vivo*. PUFA desaturation and bioconversion to carcinogenic eicosanoids as well as its oxidative modification are higher in female animals and humans [34–36]. Consistent with this notion, reports have linked countries with high PUFA intakes like Israel and the United States with a high rate of female cancers [37,38]. Therefore, we limited our animal experiments to the female sex, where any detrimental effect of PUFA could not be masked through inclusion of male-specific data. This report reports a direct relationship between 6 weeks of high PUFA intake and a loss of spontaneous activity in female mice. Although the reasons are not yet clear, it should be noted that the experimental protocol required group housing of mice (three to four mice per cage) during 6 weeks of dietary regimen, followed by 3 days of single housing to enable individual measurements of food intake, oxygen consumption and locomotor activity. It is well known that, as social creatures, single housing precipitates depression in mice and loss of spontaneous exploratory activity is a major symptom of such a phenomenon [39,40]. There is considerable evidence of clinical depression in women with a high n-6 and a low n-3 PUFA intake as that present in the corn oil diet [41,42]. It must be emphasized that, contrary to common belief, higher caloric intake by itself either does not influence or induces higher spontaneous activity in rodents [43]. A coconut-oil-based saturated fat diet leads to an increase in spontaneous activity in mice [44]. In lean humans, excess energy provision is also balanced by increased spontaneous activity and thermogenesis, at least in the short term [45].

Despite having similar food intakes and body weights between MUFA- and PUFA-fed mice, whole-body metabolism as estimated by RER was lower in corn-oil-fed mice. This implies a specific effect of n-6 PUFA on energy metabolism, primarily fat oxidation (as these mice were on a high-fat diet) that is independent of body fat mass or the fat

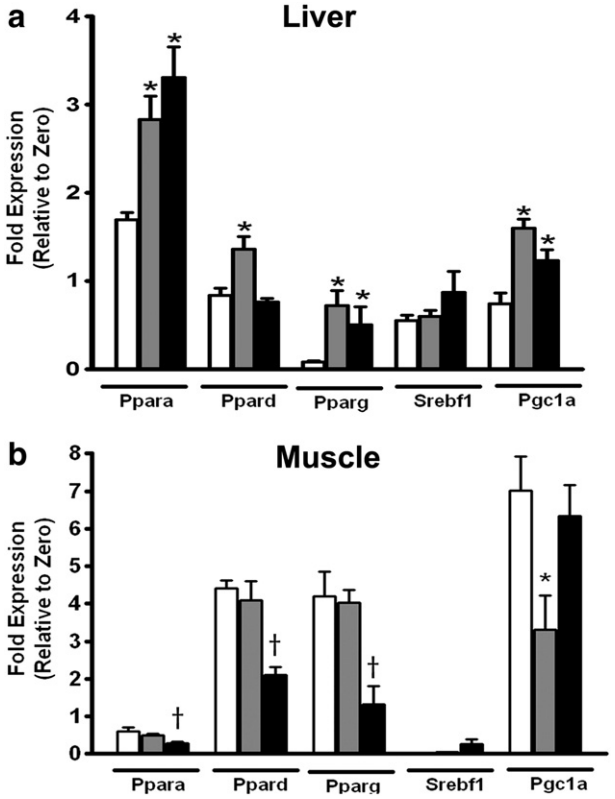


Fig. 5. Corn oil reduces fat oxidation genes in skeletal muscle but not in the liver of mice. mRNA expression of key FA-oxidation-related transcription factors from livers of mice fed different diets. (b) mRNA expression of key FA-oxidation-related transcription factors from the skeletal muscle of mice fed different diets. Data are presented as mean±S.E.M. Values were analyzed using one-way ANOVA with Tukey tests; $P<.05$, $P<.05$ ($n=6$/group). *$P<.05$ versus chow-fed mice. †$P<.05$ versus all groups of mice. PPARa, peroxisome proliferator-activated receptor alpha; PPARd, peroxisome proliferator-activated receptor delta; PPARg, peroxisome proliferator-activated receptor gamma; Srebf1, sterol regulatory element-binding protein 1; Pgc1a, peroxisome proliferator-activated receptor gamma coactivator 1-alpha.

content of the diet. Interestingly, a lack of fat oxidation with LA-rich diet has been reported earlier in humans. After a high-fat (40% energy) diet for 1 week, measurements of FA oxidation, estimated from breath sample $CO_2$, revealed lower oxidation rates in humans for dietary LA than with OA [46]. In this study, corn oil reduced expression of PPARs in the muscle, which are a major group of fatty-acid-related transcription factors. However, these factors (with the exception of PPARδ) were elevated in the liver. Both n-6 and n-3 PUFA are known to induce PPARs in the lipogenic tissues like the liver [47]; however, the role of n-6 PUFA in regulating fat-oxidation-related PPARs, especially PPARα, in the skeletal muscle remains obscure. PPARα can positively affect glucose homeostasis as demonstrated in PPARα knockout mice, which could not increase rates of fatty acid oxidation during starvation, developing characteristics of insulin resistance and diabetes [48]. However, in another study, feeding a saturated-fat-based HF diet (43% energy) actually protected PPARα-null mice from obesity or insulin resistance [49]. Thus, PUFA-mediated lowering of fat utilization in the muscle could well explain the depressed metabolic phenotype characterized by PUFA diets. Like female mice in this study, an effect of high PUFA intake on insulin resistance in human populations has been proposed earlier [50]. In this study, corn oil did not significantly increase mouse body weight compared to MUFA-fed mice; however, our dietary regimen in mice lasted 6 weeks only. With 12 weeks of high-PUFA feeding, mice do develop obesity [51] and, if continued through pregnancy, it may predispose future generations to a higher body mass as well [52].

Overall, this study provides evidence for a quantitatively important biological relationship between excess dietary PUFA intakes to loss of spontaneous activity and insulin resistance in female mice. Conversely, sustained spontaneous activity with MUFA could also be a hidden cause for established physiological benefits of the 'Mediterranean diets.' In a recent clinical study, dietary n-6 PUFA intake was identified as a cause for declining physical activity in an older population [53]. Therefore, we propose excess dietary n-6 PUFA as a novel biological risk factor which may predispose humans to a loss of physical activty. In light of such results, we strongly recommend that particular attention be paid to controlling dietary fatty acid levels, especially that of n-6 PUFA, while planning lifestyle interventions to reduce sedentary behavior and insulin resistance in susceptible populations.

## Author contributions

C.K.W. assisted in experimental design, performed metabolic cage studies and analyzed the data. A.B. performed *in vitro* experiments related to insulin resistance and edited the manuscript. J.P. performed statistical analysis and edited the manuscript. C.D. performed animal experimentations. W.T.G. provided experimental guidance through animal experiments and critically edited the manuscripts. S.G. conceived the idea, provided financial support and finalized the manuscript.

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