

# Balsam Poplar (*Populus Balsamifera*), a Traditional Eastern James Bay Cree Medicine, exerts a Limited Modulation of Intestinal Lipid Homeostasis in an Animal Model of Diet-Induced Obesity

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## Abstract

**Context:** Obesity and type 2 diabetes are at higher rates in aboriginal communities than in the general population. One reason for this condition is the cultural resistance of aboriginals to contemporary health care due to the cultural inadequacy of modern treatments for obesity and type 2 diabetes. In order to address this issue, the Canadian Institutes of Health Research Team in Aboriginal Anti-diabetic Medicines looked at 17 plants of the traditional pharmacopeia of the Cree Nations of Eastern James Bay in Canada.

**Objective:** The purpose of this present study is to examine the effect of *Populus balsamifera* on the intestinal content of various lipid species and key protein components involved in lipid metabolism.

**Methods:** Mice were exposed to a standard diet for eight weeks, a high fat diet or high fat diet with 125 mg/kg of *Populus balsamifera* integrated. Aliquots of jejunal homogenates were lipid-extracted.

**Results:** The results showed that total cholesterol content and jejunal phospholipids were not affected by the absence or the presence of *Populus balsamifera* extract. In the case of intestinal triglycerides, there was a tendency of increase with the 16-week feeding of a high fat diet and this trend seemed to vane with *Populus balsamifera* treatment. The jejunal content in fatty acids was significantly increased by the diet induce obesity treatment as compared to CHOW controls. *Populus balsamifera* treatment significantly reduced intestinal fatty acid content back towards the values observed in CHOW controls.

**Discussion and Conclusion:** This further strengthens the potential of balsam poplar extracts to be useful in the context of the high prevalence of obesity in Indigenous populations.

**Keywords:** Anti-obesity; Anti-diabetic; C57BL/6 mice; Balsam poplar; Fatty acid absorption; Aboriginal traditional medicine

## Introduction

Obesity and type II diabetes are major health problem in contemporary society and Indigenous populations are particularly affected [1,2]. For instance, the prevalence of Type II diabetes is 3 to 5 times higher among Canadian First Nations living on reserve than in the general urban population [3]. In some villages, overweight among children also reaches up to 55% (Canadian government – Healthy weights for healthy kids).

Many hypotheses have been proposed to explain overweight and obesity in Canadian First Nations: the passage from a nomadic lifestyle to a sedentary lifestyle, the presence of “thrifty genes” that allow the accumulation of fat in order to survive and the non-compliance to drug treatments bringing complications, in addition to allopathic medicine that is not well adapted to First Nation culture. In order to address these issues, notably to look toward the traditional

pharmacopeia of the Cree Nations of Eastern James Bay in Canada, the Canadian Institutes of Health Research Team in Aboriginal Anti-diabetic Medicines (CIHR-TAAM) was founded in 2003. The team now collaborates with six of the 9 villages and has identified seventeen plants with anti-diabetic proprieties.

In order to reduce health inequities between general and aboriginal populations, the CIHR-TAAM studied the anti-diabetic and anti-obesity proprieties of those seventeen identified plants using a comprehensive platform of *in vitro* bioassays and *in vivo* animal models [4]. One of these plants, *Populus balsamifera* (balsam poplar) was found to completely inhibit the differentiation of 3T3-L1 fibroblasts into mature adipocytes [5]. In subsequent studies, we used the diet-induced obesity (DIO) whereby C57BL/6 mice fed *ad libitum* with a high fat diet (HFD) developed insulin resistance and early diabetes [3]. This was associated with an increase of

total body weight, a heightened profile of blood lipids and a rise in hepatic steatosis. When DIO mice were fed *Populus balsamifera* extract along with the high fat diet, weight gain was decreased as well as hepatic steatosis [3]. Also, glycaemia and insulin levels diminished. Skeletal muscle, liver and adipose tissue were also probed with several antibodies against various components of insulin signalling, glucose and lipid metabolism. In these major insulin target tissues, *Populus balsamifera* generally improved insulin-signalling components while favoring components involved in lipid oxidation in line with the improved insulin sensitivity and reduced obesity observed systemically.

The intestine is another important organ in lipid homeostasis, being notably responsible for the packaging and delivery of lipids to the general circulation [6-8]. Therefore, in the present study, we examined the effect of *Populus balsamifera* on the intestinal content of various lipid species and key protein components involved in lipid metabolism, using tissues collected during the previous study with DIO mice [3].

## Materials and Methods

### Plant extracts

Specimens of *Populus balsamifera* L. (Salicaceae family) were collected in the Cree region of Eeyou Istchee (CEI) located in Northern Quebec, Canada. A Montreal Botanical Garden taxonomist, Dr. Alain Cuerrier, confirmed the identity of the plants. A plant sample was deposited in the Marie-Victorin herbarium of the Montreal Botanical Garden (Mis03-49). *Populus balsamifera* (commonly known as balsam poplar) was prepared as a crude 80% ethanolic extract, as previously described [5].

### Animals and diets

Four weeks old and non-diabetic C57BL/6 mice were acquired from Charles River Laboratories (Saint-Constant, QC, Canada). Mice were housed in individual cages with lighting schedules of 12 hours (light and darkness cycles). The rooms were temperature and humidity controlled and the animals had unlimited access to food and water. As described in Harbilas [5], mice were divided into groups of a dozen individuals after having been given time to acclimatize themselves to the animal facilities. A different diet was given to each of 3 groups of animals. For a period of 16 weeks, the control group (CHOW) received a standard diet composed of 18% of protein content and 4.5% crude fat, (Charles River Animal Food and Diet). For a period of 8 weeks, the 2 other groups received a high fat diet (HFD, Bio-Serv Diet #F3282, 60% energy from fat). Following this period, one of these groups continued the high fat diet for another 8 weeks while the other group received HFD into which *Populus balsamifera* extract was incorporated at 125 mg/kg, this being the most active dose seen in previous studies [3]. The Université de Montréal Ethic Committee approved all experimental protocols and protocols followed the guidelines from the Canadian Council for the Care and Protection of Animals.

### Surgical procedure

Once the experimental protocol ended, an intra-peritoneal injection of 50 mg/kg pentobarbital was used to anesthetize mice and then animals were sacrificed by ex-sanguination. Intestinal samples were collected rapidly thereafter, rinsed with PBS containing a mixture of anti-proteases (50 mM sodium fluoride, 1 mM phenyl methyl sulfonyl fluoride, 5 µg/ml and aprotinin, 5 µg/ml leupeptin).

### Lipid analyses

Aliquots of jejunal homogenates were lipid-extracted with 2:1 (vol/vol) chloroform-methanol [9,10]. Small amounts of lipid standards were added to the samples before the separation of individual lipid classes by one-dimensional thin layer chromatography (TLC) (silica gel from Eastman Kodak, Rochester, NY) as described previously [10,11]. The

non polar solvent system was 80:20:3 (vol/vol/vol) hexane-diethylether-glacial acetic acid. The lipid concentrations of the separated fractions were measured enzymatically by commercial kits (Boehringer Mannheim, Montreal) after elution with exan and evaporation.

### Western blot analysis

To assess the protein expression of various proteins, the jejunal tissue was homogenized in lysis buffer [50 mM HEPES (pH 7.5), 150 mM sodium chloride, 1.5 mM magnesium chloride, 1 mM EGTA, 1% TritonX-100, 50 mM sodium fluoride, 10% glycerol, 1 mM phenylmethylsulfonyl fluoride, 5 µg/ml Aprotinin, 5 µg/ml leupeptin, and 1 mM sodium orthovanadate], followed by centrifugation at 13,000 rpm for 10 min and prepared for Western blotting as described previously [12]. The following antibodies were employed: anti-FAS, anti ACC, anti-pACC anti-CPT1 from Santa Cruz Biotechnology (Santa Cruz, CA).

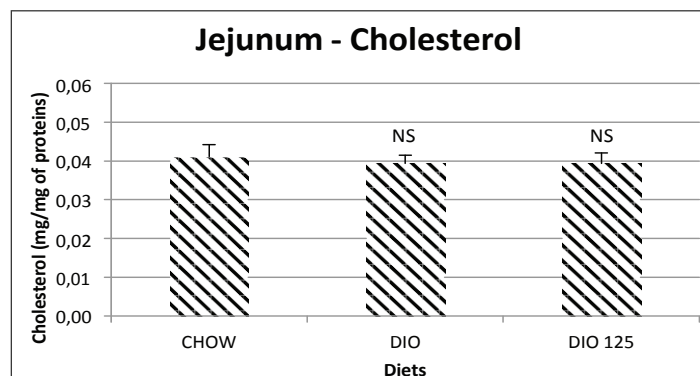
The Bradford assay [Bio-Rad Laboratories (Canada) Ltd., Mississauga, Ontario, Canada] was used to determine protein concentration. Proteins were denatured in sample buffer containing SDS and β-mercaptoethanol, separated on a 7.5% SDS-PAGE gel, and blotted onto nitrocellulose membranes. Nonspecific binding sites of the membranes were blocked using 5% defatted milk proteins. Reactions took place by the addition of primary antibodies directed against targeted proteins. Reaction was revealed with species-specific horseradish peroxidase-conjugated secondary antibody and enhanced chemiluminescence reagent (PerkinElmer, Waltham, MA). β-actin was used as an internal control to confirm equal loading protein on SDS-PAGE. Blots were developed, and proteins were quantified using a Hewlett-Packard scanner equipped with a transparency adaptor and UN-SCAN-IT (Silk Scientific Inc., Orem, UT) software.

### Statistical analysis

A one-way analysis of variance (ANOVA) was used to analyze data using the software Prism 5.0. Statistical significance was set at  $p < 0.05$ .

## Results

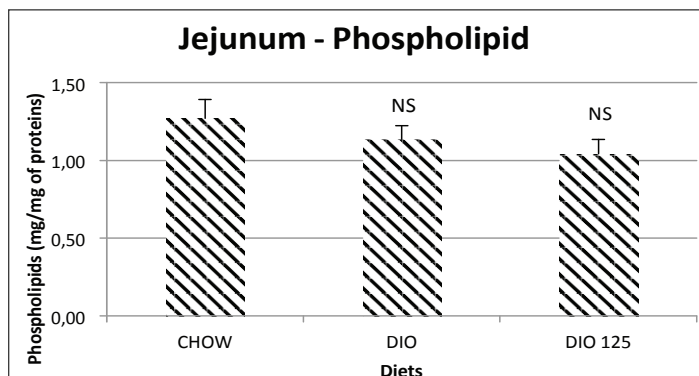
Intestinal lipid parameters were measured in jejunal segments obtained from CHOW controls, DIO controls (16 weeks on HFD) and DIO animals receiving 125 mg/kg of *Populus balsamifera* extract for the last 8 weeks of HFD treatment. As illustrated in figure 1, intestinal total cholesterol content was not affected by the DIO treatment in the absence or presence of *Populus balsamifera* extract. Similar results were obtained for jejunal phospholipids (Figure 2).



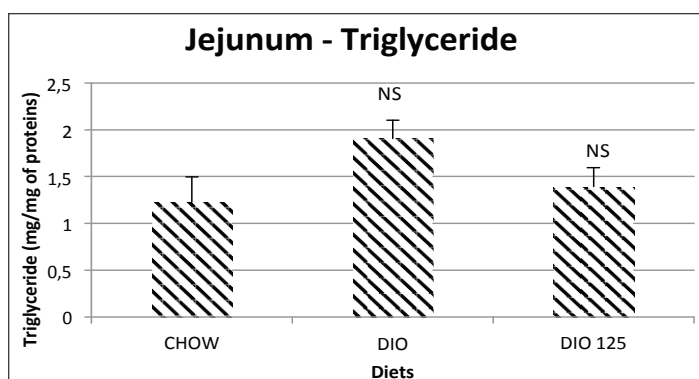
**Figure 1:** Jejunal total cholesterol is not modified by high fat diet or *P. Balsamifera* treatment. Jejunal total cholesterol was measured as described in Materials and Methods and expressed as mg per mg of jejunal protein. N.S. Not significantly different from CHOW controls by one-way ANOVA, N=8 per group.

In the case of intestinal triglycerides, there was a tendency of increase with the 16-week feeding of a HFD and this trend seemed to vane with *Populus balsamifera* treatment (Figure 3). However, data variability precluded any statistical significance in these trends. On the other and, the jejunal content in fatty acids was significantly increased by the DIO treatment as compared to CHOW controls ( $p < 0.05$ ; Figure 4). *Populus balsamifera* treatment significantly reduced intestinal fatty acid content back toward values observed in CHOW controls ( $p < 0.05$  versus DIO, N.S. versus CHOW; (Figure 4).

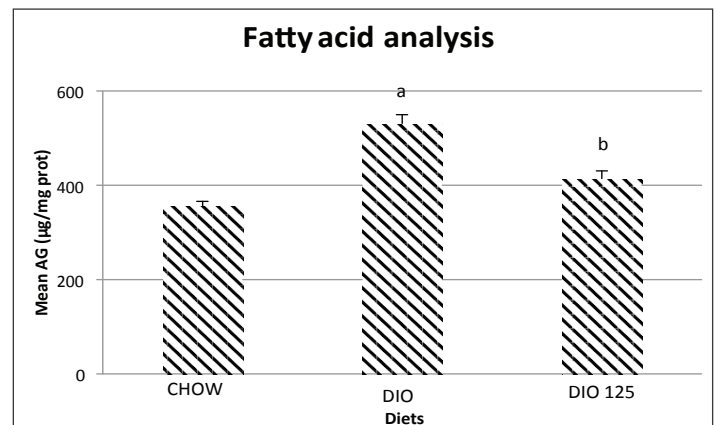
To elucidate the mechanisms underlying changes in intestinal lipid handling, we used Western blot analysis to measure the expression of three key lipid metabolizing enzymes, namely fatty acid synthase (FAS), carnitine palmytoyltransferase-1 (CPT-1) and acetyl co-Acarboxylase (ACC). As shown in figure 5, the DIO dietary regimen induced a significant decrease in the content of intestinal FAS ( $p < 0.05$  versus CHOW) and *Populus balsamifera* treatment had a tendency to partially normalize the values (N.S. versus either CHOW or DIO control groups). On the other hand, CPT-1 was reduced in both DIO and *Populus balsamifera*-treated mice as compared to CHOW control animals ( $p < 0.05$ ; (Figure 6). In the case of the phosphorylated (inactivated) form of ACC (pACC), it was again significantly diminished by the DIO regimen ( $p < 0.05$  versus CHOW; (Figure 7). However, *Populus balsamifera* treatment completely returned levels of pACC back to levels found in CHOW control mice (N.S. versus CHOW, (Figure 7).



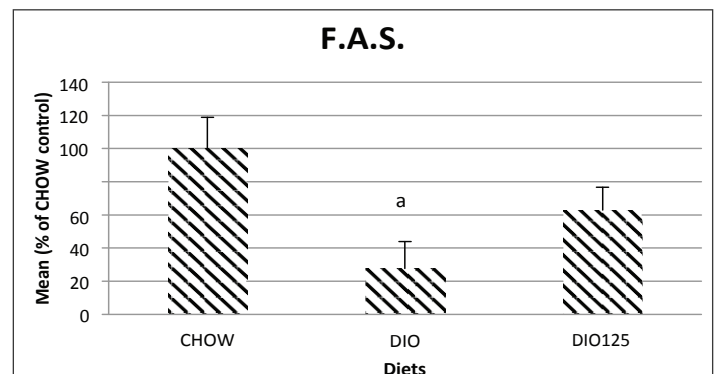
**Figure 2:** Lack of effect of high fat diet or *P. Balsamifera* treatment on jejunal phospholipid content. Jejunal phospholipid content was determined as described in Materials and Methods and is expressed as mg per mg of jejunal protein. N.S. Not significantly different from CHOW controls by one-way ANOVA, N=8 per group.



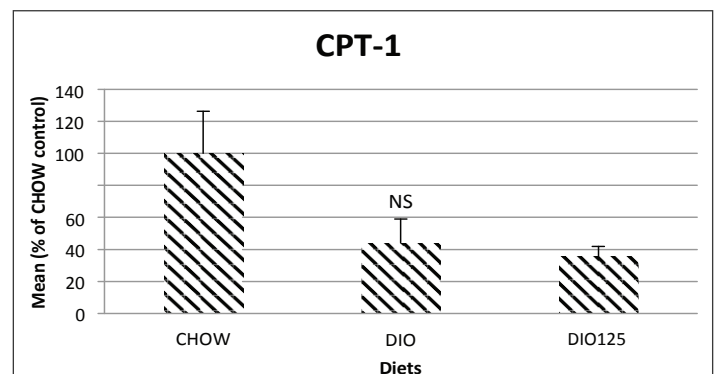
**Figure 3:** Jejunal triglycerides are not significantly affected by high fat diet or *P. Balsamifera* treatment. Jejunal triglycerides were measured as described in Materials and Methods and expressed as mg per mg of jejunal protein. N.S. Not significantly different from CHOW controls or from DIO group by one-way ANOVA, N=8 per group.



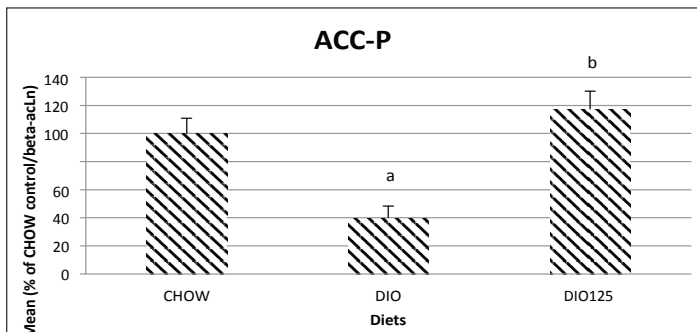
**Figure 4:** High fat diet increases jejunal fatty acid content and this effect is reversed by *P. Balsamifera* treatment. Jejunal fatty acid content was measured as described in Materials and Methods and is expressed as µg per mg of jejunal protein. Significantly different from CHOW controls (a;  $p < 0.05$ ) or from DIO group (b;  $p < 0.05$ ) by one-way ANOVA, N=8 per group.



**Figure 5:** Jejunal fatty acid synthase is reduced by high fat diet treatment but not when the latter is combined with *P. Balsamifera* treatment. Jejunal FAS was assayed by Western blot analysis as described in Materials and Methods and is expressed as a percentage of densitometric values obtained for CHOW control animals. Significantly different from CHOW controls (a;  $p < 0.05$ ) by one-way ANOVA. N=8 per group.



**Figure 6:** Jejunal CPT-1 is not significantly affected by high fat diet or *P. Balsamifera* treatment. Jejunal CPT-1 were measured by Western blot analysis as described in Materials and Methods and is expressed as a percentage of densitometric values obtained for CHOW control animals. N.S. Not significantly different from CHOW controls or from DIO group by one-way ANOVA, N=8 per group.



**Figure 7:** High fat diet decreases jejunal phosphorylated ACC and this effect is reversed by *P. Balsamifera* treatment. Jejunal phosphorylated ACC was measured by Western blot analysis as described in Materials and Methods and is expressed as a percentage of densitometric values obtained for CHOW control animals. Significantly different from CHOW controls (a;  $p < 0.05$ ) or from DIO group (b;  $p < 0.05$ ) by one-way ANOVA,  $N = 8$  per group.

## Discussion

Our team, the CIHR-TAAM, has been working since 2003 with the Cree First Nations of Eastern James Bay in Canada to rigorously study potential anti-diabetic and anti-obesity plants from their traditional pharmacopeia. While screening for glitazone-like activity in differentiating 3T3-L1 adipocytes, we discovered that the ethanol extract of balsam poplar, *Populus balsamifera*, completely abrogated adipogenesis, thereby suggesting a possible anti-obesity effect [5,13]. We have thus more recently carried out an *in vivo* study using the DIO mouse model to examine the effects of balsam poplar on body weight and metabolic parameters. We reported that blood lipid parameters of DIO mice were modulated in the following manner: LDL levels were more than tripled, HDL increased by about 50% and total cholesterol doubled as compared to CHOW controls, whereas circulating triglycerides remained unchanged [3]. Although it reduced body weight and improved glucose homeostasis as well as insulin sensitivity, *Populus balsamifera* treatment did not have any significant effect on blood lipid parameters. However, it reduced hepatic steatosis and corresponding liver triglyceride content as compared to DIO control animals [3]. The intestine is another important organ implicated in body lipid homeostasis [14]. Since we had also collected intestinal samples at animal sacrifice in our previous work [3], the aim of the current study was to evaluate the effects of the DIO protocol and of *Populus balsamifera* treatment on jejunal lipid content and on key proteins implicated in intestinal lipid homeostasis.

The results presented herein demonstrate that the DIO regimen of feeding a HFD for 16 weeks had little effect on jejunal total cholesterol and phospholipids while it tended to elevate intestinal triglycerides, albeit not in a statistically significant manner. In contrast, the feeding of the HFD increased jejunal fatty acid content significantly.

This effect was reversed by *Populus balsamifera* treatment while other lipid parameters were not significantly affected, although there was a trend for the plant to reverse the DIO-induced reduction of FAS expression. Together with our previously reported results on systemic lipid parameters in DIO mice [3], the present study suggests that despite a large dietary lipid load, little cholesterol, phospholipids and triglycerides seem to accumulate in intestinal cells. In contrast, the increase in jejunal fatty acids may stem from an overload of the lipid producing machinery of enterocytes. Indeed, our results showed that the expression of FAS was diminished in DIO animals. This would limit fatty acid synthesis in accordance with the large dietary supply of the latter. Similarly, the expression of CPT-1, a key enzyme of lipid beta-oxidation, was also reduced. This would limit the

oxidation of fatty acids and contribute further to their observed cellular accumulation. On the other hand, ACC phosphorylation (pACC) was decreased in DIO animals and this indicates an increase in ACC activity. Normally, this should supply more malonyl-CoA for fatty acid synthesis, but FAS was found to be diminished. Malonyl-CoA also inhibits CPT-1, which is consistent with our current observations. One possibility to explain the reduced pACC is that jejunal AMP-dependent kinase activity, which normally phosphorylates and inhibits ACC, may be diminished in DIO animals. However, the phosphorylated and active form of AMPK in skeletal muscle was not significantly different between DIO mice and CHOW controls in our previous work [3]. Further studies will be necessary to clarify this point.

Without affecting circulating lipid parameters in DIO mice, we previously reported that *Populus balsamifera* treatment reduced body weight, improved glycemia and insulinemia while favouring insulin signalling and fat oxidative components in liver and adipose tissue. In jejuna segments in the current study, *Populus balsamifera* treatment had two major effects. It reduced the cellular fatty acid content and increased ACC phosphorylation. Both of these components could be related to an increase in AMPK activity leading to lipid “wastage”, as previously suggested [3]. Indeed, we have also previously reported that balsam poplar more than doubled AMPK activation in cultured hepatocytes [15]. However, since the reduced expression of CPT-1 was not reversed by *Populus balsamifera* treatment, it is hard to envisage that beta oxidation of fatty acids may have been improved. Other oxidative pathways, such as omega-oxidation of fatty acids, could be increased in the intestine, as was seen with dietary lipid interventions in non-obese C57BL/6J mice [16].

## Conclusion

In summary, the effects of *Populus balsamifera* treatment on jejunal segments of DIO mice were limited to reducing fatty acid content and ACC activation (through increased phosphorylation). Nonetheless, such actions should be beneficial in the face of high fat diets and obesity. This further strengthens the potential of balsam poplar extracts to be useful in the context of the high prevalence of obesity in Indigenous populations, such as the Cree of Eeyou Istchee. *Populus balsamifera* traditional preparations should thus be studied further, notably in clinical settings, in such populations.

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## Disclosure

The authors declare no conflict of interest.

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