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## Multiple-stressor effects in an apex predator: combined influence of pollutants and sea ice decline on lipid metabolism in polar bears

Sabrina Tartu<sup>1</sup>, Roger Lille-Langøy<sup>2</sup>, Trond R. Størseth<sup>3</sup>, Sophie Bourgeon<sup>1,4</sup>, Anders Brunsvik<sup>3</sup>, Jon Aars<sup>1</sup>, Anders Goksøyr<sup>2</sup>, Bjørn Munro Jenssen<sup>5</sup>, Anuschka Polder<sup>6</sup>, Gregory W. Thiemann<sup>7</sup>, Vidar Torget<sup>5</sup> & Heli Routti<sup>1</sup>

There is growing evidence from experimental and human epidemiological studies that many pollutants can disrupt lipid metabolism. In Arctic wildlife, the occurrence of such compounds could have serious consequences for seasonal feeders. We set out to study whether organohalogenated compounds (OHCs) could cause disruption of energy metabolism in female polar bears (*Ursus maritimus*) from Svalbard, Norway (n = 112). We analyzed biomarkers of energy metabolism including the abundance profiles of nine lipid-related genes, fatty acid (FA) synthesis and elongation indices in adipose tissue, and concentrations of lipid-related variables in plasma (cholesterol, high-density lipoprotein, triglycerides). Furthermore, the plasma metabolome and lipidome were characterized by low molecular weight metabolites and lipid fingerprinting, respectively. Polychlorinated biphenyls, chlordanes, brominated diphenyl ethers and perfluoroalkyl substances were significantly related to biomarkers involved in lipid accumulation, FA metabolism, insulin utilization, and cholesterol homeostasis. Moreover, the effects of pollutants were measurable at the metabolome and lipidome levels. Our results indicate that several OHCs affect lipid biosynthesis and catabolism in female polar bears. Furthermore, these effects were more pronounced when combined with reduced sea ice extent and thickness, suggesting that climate-driven sea ice decline and OHCs have synergistic negative effects on polar bears.

Since many chemicals are lipid-soluble, it is relevant to investigate whether their concurrent and continuous presence in adipose tissues may be harmful<sup>1</sup>. A major role of adipose tissue is to store ingested energy in the form of triglycerides during periods of energy excess and make it available during periods of energy deprivation<sup>2</sup>. Recently it has been recognized that adipose tissue is an endocrine organ involved in functions such as immune response, inflammation, reproduction and metabolism<sup>3,4</sup>. In mammals, the regulation of lipid metabolism is the result of the concomitant action of the central nervous system and several organs such as the liver, muscles and adipose tissue, via hormonal messengers. In white adipocytes, fatty acid (FA) transport, synthesis, uptake and lipid hydrolysis are controlled by a set of genes mostly regulated by “the master regulator of adipogenesis”: the nuclear receptor peroxisome-proliferator activated receptor gamma (PPARG)<sup>5</sup>. Several factors interact with PPARG to facilitate lipid metabolism (e.g. lipid synthesis, storage and hydrolysis). These include, for example, the PPARG-coactivator-1 (PGC1), which increases the expression of genes encoding respiratory chain proteins, enzymes of FA oxidation, and causing white adipocytes to acquire features of brown adipocytes<sup>6</sup>. Another factor interacting with PPARG is the sterol regulatory element-binding protein 1 (SREBP1)<sup>7</sup>, which leads to increased FA synthesis and their storage as triglycerides in adipocytes and peripheral tissues<sup>8–10</sup>. *Cluster of differentiation 36* (CD36) and *FA binding protein 4* (FABP4) are PPARG target genes whose gene products are involved in FA uptake and transport<sup>10</sup>. Moreover, the FA synthase (FASN) induces FA synthesis and produces ligands to PPARG<sup>10,11</sup>.

<sup>1</sup>Norwegian Polar Institute, Fram Centre, NO-9296, Tromsø, Norway. <sup>2</sup>University of Bergen, Department of Biology, NO-5020, Bergen, Norway. <sup>3</sup>SINTEF, Trondheim, Norway. <sup>4</sup>UiT-The Arctic University of Norway, Department of Arctic and Marine Biology, NO-9019, Tromsø, Norway. <sup>5</sup>Norwegian University of Science and Technology, Department of Biology, NO-7491, Trondheim, Norway. <sup>6</sup>Norwegian University of Life Science, Campus Adamstua, NO-0033, Oslo, Norway. <sup>7</sup>York University, Faculty of Environmental Studies, Toronto, ON, M3J 1P3, Canada. Correspondence and requests for materials should be addressed to S.T. (email: [tartu.sabrina@gmail.com](mailto:tartu.sabrina@gmail.com))

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The breakdown of triglycerides to free FAs (FFAs) and diacylglycerol occurs via the action of the patatin-like phospholipase domain-containing protein 2 (PNPLA2), another PPARG target gene product, and the second hydrolysis step (break down of diacylglycerol to monoacylglycerol and FFAs) is catalyzed by lipase E (LIPE, also known as hormone sensitive lipase) and other lipases<sup>12</sup>. Adiponectin (ADIPOQ), a protein hormone regulated by PPARG, sensitizes insulin and stimulates energy metabolism in tissues<sup>13,14</sup>.

Several experimental and epidemiological studies on mammals have indicated that organohalogenated compounds (OHCs) such as polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs) and poly- and perfluoroalkyl substances (PFASs) affect lipid metabolism<sup>15–19</sup>. Arctic top predators, such as polar bears (*Ursus maritimus*), are among the most polluted species in the world<sup>20</sup> and have evolved large body lipid stores in response to seasonal variation in prey availability and cold temperatures<sup>21</sup>. Polar bears have a unique metabolism that enables them to deal with a lipid-rich diet and to efficiently accumulate adipose tissue during periods of food abundance<sup>22,23</sup>. Within a few months (generally from April to July), polar bears feed actively and can accumulate up to 50% of their body mass as lipids, through consumption of seal blubber during the ringed seal (*Pusa hispida*) pupping and moulting periods<sup>24</sup>. These energy stores are metabolized during periods of food scarcity or reproductive fasting. For instance, polar bears frequently fast in autumn if stranded in areas without access to sea ice, and prey encounter is thought to be low in winter<sup>25</sup>. Female polar bears can sustain a 4–8 month fasting period (late autumn/early winter to the following spring) during denning, associated with parturition and lactation<sup>26</sup>. The reproductive success of female polar bears thus directly depends on their lipid stores<sup>24</sup>.

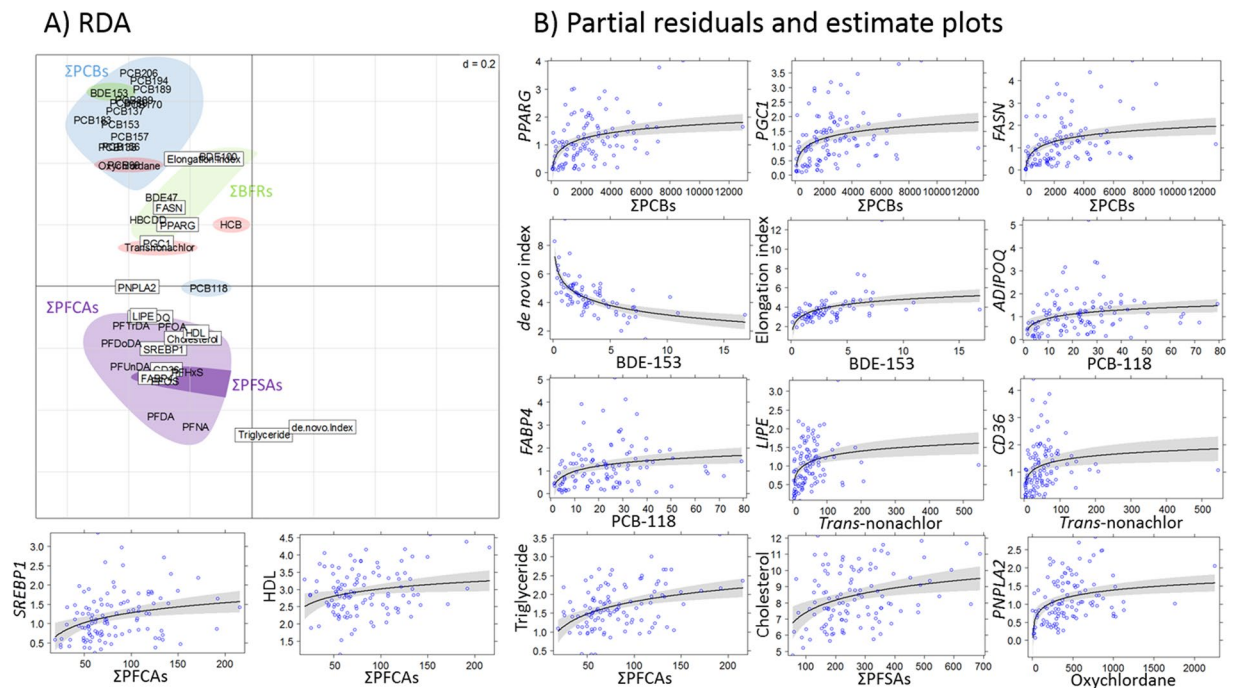
There is a lack of information on how pollutants may affect energy metabolism in free-ranging polar bears. An appropriate response to seasonal feeding is to accumulate triglycerides in adipose tissue when food is available and release this energy in the form of FAs and glycerol in times of food scarcity. Routti *et al.* (2016) recently showed that lipophilic pollutants (e.g. PCBs, OCPs and brominated flame retardants: BFRs) stored in polar bear adipose tissue modulate the polar bear PPARG function and the differentiation of polar bear stem cells into adipocytes<sup>27</sup>. Furthermore, pollutants are suspected to impair thyroid hormone concentrations in polar bears<sup>28–32</sup> and thyroid hormones are known to be involved in the synthesis, mobilization and degradation of lipids<sup>33,34</sup>. Improved knowledge of the effects of pollutants on energy metabolism is crucial to understanding how polar bears will respond to ongoing habitat loss associated with global warming<sup>35</sup>. Sea ice decline has been documented to result in longer periods of fasting, reduced foraging opportunities, lower body condition, decreased access to denning areas and lower survival of cubs<sup>36–38</sup>. Furthermore, declining sea ice has contributed to increased concentrations of lipophilic pollutants in polar bears due to a decrease in body condition<sup>39</sup>. Impaired lipid metabolism caused by pollutant exposure may have significant consequences on polar bear physiology and reproduction<sup>35</sup>. Within the circumpolar habitat of polar bears, the Barents Sea has been experiencing the fastest loss of sea ice extent over the past decades<sup>40</sup>, and polar bears from that area are among the most polluted Arctic wildlife populations with respect to OHCs<sup>20,41–45</sup>. The combination of these two factors may put Barents Sea polar bears at especially high risk to multi-stress effects by pollutants and climate change.

The main goal of the present study was to investigate the effects of OHC exposure on energy metabolism in Barents Sea polar bears at multiple levels. In addition, we described how energy biomarkers varied according to nutritional state (feeding or fasting individuals) and we investigated whether sea ice conditions interact with the effects of OHCs on energy metabolism in polar bears. Specifically, we investigated whether the response of energy metabolism to pollutant exposure differed when sea ice was less extended and bears were under nutritional stress compared to more “seasonally adequate” sea ice conditions<sup>39</sup>. The biomarkers of energy metabolism included targeted analyses (i.e. gene transcription and FA metabolism in adipose tissue, and concentrations of lipid related variables in plasma), and non-targeted end-points such as plasma metabolome and lipidome, which are considered as final downstream products of gene transcription.

## Results

**Targeted biomarkers of energy metabolism.** We assumed that most females captured in April would be in a feeding state whereas most of those captured in September would be in a fasting state. In female polar bears, which were in a feeding state, we measured higher expressions of *PPARG*, *PGC1* and *FASN* transcript levels (Table S1) in adipose tissue as compared to fasting bears. Fasting females had increased *de novo* FA synthesis, decreased FA elongation index and higher plasma concentrations of triglycerides (Table S1). Several variables related to lipid metabolism remained unchanged between the two metabolic states (feeding or fasting), including *PNPLA2*, *LIPE*, *ADIPOQ*, *CD36* and *FABP4* transcript levels and concentrations of cholesterol and high-density lipoprotein (HDL) (Table S1).

The pollutants included in the redundancy analyses (RDA) explained 62% of the variation of the biomarkers of energy metabolism, and the RDA model was highly significant based on Monte-Carlo test (1000 replicates, RV coefficient = 0.49,  $p < 0.001$ ). According to their proximity on the RDA plot, some pollutants were summed to reduce the number of predictors used in generalized linear mixed models (GLMMs), resulting in nine pollutant predictors (Fig. 1A). Oxychlordane, *trans*-nonachlor, hexachlorobenzene (HCB), PCB-118 and BDE-153 were considered individually, whereas the remaining compounds were summed as follows:  $\Sigma_{13}$ PCBs (PCB-99, -137, -138, -153, -156, -157, -170, -180, -183, -189, -194, -206 and -209),  $\Sigma_3$  brominated flame retardants (BFRs: BDE-47, -100, hexabromocyclododecane),  $\Sigma_6$  perfluoroalkyl carboxylates (PFCA with carbon chain length of 8 to 13) and  $\Sigma_2$  perfluoroalkyl sulfonates (PFSA with 6 and 8 carbons). Pollutant concentrations (mean ng/g wet weight  $\pm$  SD) in polar bear adipose tissue were  $2607 \pm 2137$  for  $\Sigma_{13}$ PCBs,  $22.8 \pm 15.9$  for PCB-118,  $62.2 \pm 75.9$  for HCB,  $460 \pm 349$  for oxychlordane,  $53.0 \pm 62.5$  for *trans*-nonachlor,  $22.1 \pm 13.8$  for  $\Sigma_3$ BFRs and  $3.37 \pm 3.08$  for BDE-153. Plasma concentrations (ng/g wet weight) of  $\Sigma_2$ PFSA and  $\Sigma_6$ PFCA were  $264 \pm 130$  and  $81.7 \pm 38.0$ , respectively.



**Figure 1.** Relationships between biomarkers of energy metabolism and pollutants. (A) Redundancy analysis (RDA) loading plot ( $n = 80$ ) and (B) partial residuals and estimate plots obtained from mixed models ( $n = 111$ ). Plasma and fat samples are from Svalbard female polar bears captured during spring (April) and autumn (September) 2012 and 2013. For the RDA (A), boxed labels are response variables and unboxed labels are predictors. (B) Dots are the partial residuals, the solid line is the parameter estimate and the grey area represents its 95% confidence interval derived from mixed models. Pollutant concentrations are in ng/g wet weight, transcript levels are in arbitrary units, lipid parameters in mmol/L.

Relationships between biomarkers of energy metabolism and pollutants were consistent between RDA and model averaging estimates (Fig. 1A,B, Table 1). To identify the strongest predictors, we focused on the top models explaining the variation in biomarkers of energy metabolism ( $\Delta AIC_c < 2$ ). The three most competitive models and model averaged estimates for these models are given in Table 1. Overall, three major groups of pollutants explained most of the variation of biomarkers of energy metabolism. Specifically,  $\Sigma$ PCBs, BDE-153 and oxychlorthane were the strongest predictors for *PPARG*, *PGC1*, *FASN*, *PNPLA2* transcript levels and FA indexes (*de novo* synthesis and elongation). *Trans-nonachlor* and PCB-118 were the strongest predictors for *LIPE*, *ADIPOQ*, *CD36* and *FABP4* transcript levels.  $\Sigma$ PFASs and  $\Sigma$ PFASs were among the strongest predictors for *SREBP1* transcript levels, and, concentrations of cholesterol, HDL and triglycerides (Table 1). Generally, pollutants predicted increases of biomarkers of energy metabolism, except for BDE-153, which predicted a decrease of *de novo* FA synthesis (Fig. 1B, Table 1).

**Non-targeted biomarkers of energy metabolism: Metabolome and lipidome.** The partial least square (PLS) scores for polar bear metabolome significantly clustered according to season (MANOVA, Pillai trace test,  $F = 31.7$ ,  $p < 0.001$ , Fig. 2A). The loadings (Figure S2) indicated that glucose and lactate were the main metabolites driving seasonal metabolome segregation with higher concentrations of glucose and lactate in feeding (April) compared to fasting (September) females (see Table S1 for estimates and 95% CI according to season). Other metabolites that had less influence on the metabolome segregation were not quantifiable due to misalignment of peaks, which may have resulted from the presence of plastic polymers in the sampling tubes or heparin in the plasma samples<sup>46,47</sup>. For the lipidome, the PLS scores also clustered according to season (MANOVA, Pillai trace test,  $F = 53.09$ ,  $p < 0.001$ ).

Plasma  $\Sigma$ PFCA concentrations predicted a decrease of lactate concentrations, whereas OHCs did not influence glucose concentrations in plasma (Table 1). A PLS discriminant analysis correlation plot on the lipidome and pollutants clustered pollutants mainly into three groups, one containing PCBs, oxychlorthane and BFRs, another one containing PFASs (Fig. 2B), and the third cluster containing *trans-nonachlor*, PCB-118 and HCB. The third cluster slightly correlated to a lipid with a mass of 866.7 m/z for which it was not possible to assign the mass of any specific lipid due to low abundance in Fourier transform ion cyclotron resonance (FTI-CR). A lipid with a mass of 797.6 m/z was found to correlate with the PCBs, oxychlorthane and BFRs cluster using the time-of-flight mass-spectrometry data, which was assigned as the isotope peak of 796.6 using the FT-ICR coupled to mass spectrometry (glycerophosphocholine 37:3).

**Declining sea ice exacerbates the effects of pollutants on polar bear energy metabolism.** During the three periods where availability to sea ice was highest (i.e. April and September 2012 and September 2013), the metabolic responses of female polar bears were not different between females with high versus low

Response variable	Predictor	Three most competitive models					Conditional average estimates and 95% CI	
		df	log Likelihood	AICc	$\Delta$ AICc	weight	Intercept	Predictor
PPARG	$\Sigma$ PCBs	5	<b>-122.16</b>	<b>254.89</b>	<b>0</b>	<b>0.69</b>	-0.69 [-2.68; 1.29]	<b>0.32 [0.18; 0.47]</b>
	BDE-153	5	-123.22	257.02	2.13	0.24		<b>0.33 [0.17; 0.48]</b>
	Oxychlorthane	5	-124.61	259.8	4.92	0.06		<b>0.30 [0.15; 0.45]</b>
PGC1	$\Sigma$ PCBs	5	<b>-127.19</b>	<b>264.96</b>	<b>0</b>	<b>0.52</b>	-0.38 [-2.21; 1.45]	<b>0.30 [0.15; 0.45]</b>
	<b>BDE-153</b>	5	<b>-127.96</b>	<b>266.5</b>	<b>1.54</b>	<b>0.24</b>		<b>0.30 [0.14; 0.47]</b>
	Oxychlorthane	5	-128.31	267.19	2.23	0.17		<b>0.29 [0.13; 0.45]</b>
FASN	$\Sigma$ PCBs	5	<b>-151.86</b>	<b>314.29</b>	<b>0</b>	<b>0.52</b>	-0.62 [-2.99; 1.75]	<b>0.37 [0.18; 0.55]</b>
	<b>BDE-153</b>	5	<b>-152.34</b>	<b>315.25</b>	<b>0.96</b>	<b>0.32</b>		<b>0.38 [0.19; 0.58]</b>
	Oxychlorthane	5	-153.07	316.72	2.43	0.15		<b>0.35 [0.16; 0.55]</b>
PNPLA2	<b>Oxychlorthane</b>	5	<b>-93.65</b>	<b>197.87</b>	<b>0</b>	<b>0.58</b>	-0.21 [-1.15; 0.74]	<b>0.24 [0.12; 0.36]</b>
	$\Sigma$ PCBs	5	<b>-94.51</b>	<b>199.6</b>	<b>1.73</b>	<b>0.25</b>		<b>0.22 [0.11; 0.34]</b>
	Trans-nonachlor	5	-95.45	201.47	3.6	0.1		<b>0.21 [0.09; 0.33]</b>
LIPE	<b>Trans-nonachlor</b>	5	<b>-85.14</b>	<b>180.86</b>	<b>0</b>	<b>0.84</b>	0.29 [-0.14; 0.73]	<b>0.21 [0.10; 0.31]</b>
	PCB-118	5	-87.39	185.36	4.5	0.09		<b>0.20 [0.08; 0.33]</b>
	Oxychlorthane	5	-88.38	187.34	6.48	0.03		<b>0.17 [0.06; 0.28]</b>
ADIPOQ	<b>PCB-118</b>	5	<b>-121.6</b>	<b>253.78</b>	<b>0</b>	<b>0.48</b>	0.25 [-0.51; 1.02]	<b>0.26 [0.09; 0.44]</b>
	<b>Trans-nonachlor</b>	5	<b>-122.21</b>	<b>254.98</b>	<b>1.21</b>	<b>0.26</b>		<b>0.22 [0.07; 0.36]</b>
	Oxychlorthane	5	-123.1	256.76	2.99	0.11		<b>0.20 [0.05; 0.36]</b>
CD36	<b>Trans-nonachlor</b>	5	<b>-133.63</b>	<b>277.83</b>	<b>0</b>	<b>0.5</b>	0.28 [-0.42; 0.98]	<b>0.25 [0.09; 0.41]</b>
	<b>PCB-118</b>	5	<b>-133.92</b>	<b>278.42</b>	<b>0.59</b>	<b>0.37</b>		<b>0.29 [0.09; 0.48]</b>
	Oxychlorthane	5	-136.26	283.1	5.27	0.04		<b>0.18 [0.01; 0.35]</b>
FABP4	<b>PCB-118</b>	5	<b>-144.18</b>	<b>298.92</b>	<b>0</b>	<b>0.52</b>	0.17 [-0.69; 1.04]	<b>0.33 [0.12; 0.55]</b>
	<b>Trans-nonachlor</b>	5	<b>-144.57</b>	<b>299.72</b>	<b>0.79</b>	<b>0.35</b>		<b>0.27 [0.09; 0.45]</b>
	$\Sigma$ PFCAs	5	-146.84	304.26	5.34	0.04		<b>0.45 [0.04; 0.86]</b>
SREBP1	$\Sigma$ PFCAs	5	<b>-92.8</b>	<b>196.17</b>	<b>0</b>	<b>0.44</b>	0.11 [-1.28; 1.50]	<b>0.37 [0.11; 0.64]</b>
	<b>Trans-nonachlor</b>	5	<b>-93.19</b>	<b>196.96</b>	<b>0.79</b>	<b>0.29</b>		<b>0.15 [0.04; 0.27]</b>
	$\Sigma$ PFSAs	5	-94.55	199.68	3.51	0.08		<b>0.27 [0.02; 0.52]</b>
de novo synthesis index	<b>BDE-153</b>	5	<b>-111.15</b>	<b>233.1</b>	<b>0</b>	<b>0.99</b>	5.35 [4.44; 6.26]	<b>-0.96 [-1.27; -0.65]</b>
	$\Sigma$ PCBs	5	-116.38	243.55	10.45	0.01		<b>-0.89 [-1.24; -0.53]</b>
	Oxychlorthane	5	-123.81	258.41	25.31	0		<b>-0.74 [-1.16; -0.31]</b>
Elongation index	<b>BDE-153</b>	5	<b>-135</b>	<b>280.78</b>	<b>0</b>	<b>0.98</b>	3.04 [1.84; 4.23]	<b>0.73 [0.32; 1.14]</b>
	$\Sigma$ PCBs	5	-138.86	288.5	7.72	0.02		<b>0.58 [0.12; 1.04]</b>
	Oxychlorthane	5	-142.02	294.83	14.05	0		<b>0.37 [-0.15; 0.9]</b>
Cholesterols	$\Sigma$ PFSAs	5	<b>-205.21</b>	<b>420.99</b>	<b>0</b>	<b>0.63</b>	3.27 [-1.17; 7.71]	<b>1.08 [0.40; 1.77]</b>
	$\Sigma$ PFCAs	5	<b>-206.04</b>	<b>422.66</b>	<b>1.67</b>	<b>0.27</b>		<b>1.08 [0.35; 1.82]</b>
	BDE-153	5	-207.88	426.33	5.34	0.04		<b>0.38 [0.04; 0.73]</b>
HDL	<b>BDE-153</b>	5	<b>-105.53</b>	<b>221.64</b>	<b>0</b>	<b>0.33</b>	2.32 [1.02; 3.61]	<b>0.15 [0.02; 0.29]</b>
	$\Sigma$ PFCAs	5	<b>-105.99</b>	<b>222.56</b>	<b>0.91</b>	<b>0.21</b>		<b>0.30 [0.02; 0.59]</b>
	$\Sigma$ PFSAs	5	-106.63	223.84	2.2	0.11		<b>0.24 [-0.02; 0.50]</b>
Triglycerides	$\Sigma$ PFCAs	5	<b>-84.58</b>	<b>179.74</b>	<b>0</b>	<b>0.84</b>	0 [-1.99; 1.98]	<b>0.47 [0.24; 0.70]</b>
	BDE-153	5	-86.83	184.23	4.49	0.09		<b>-0.19 [-0.31; -0.08]</b>
	$\Sigma$ PCBs	5	-87.73	186.04	6.3	0.04		<b>-0.17 [-0.28; -0.06]</b>
Glucose	Null model	4	-284.64	577.67	0	0.17	2.58 [-2.17; 0.7]	<b>0.39 [-2.36; 0.77]</b>
	PCB-118	5	-284.03	578.64	0.97	0.11		<b>-0.73 [-0.34; 0.98]</b>
	$\Sigma$ PFSAs	5	-284.04	578.66	0.99	0.11		<b>-0.79 [-0.33; 0.89]</b>
Lactate	$\Sigma$ PFCAs	5	<b>38.71</b>	<b>-66.84</b>	<b>0</b>	<b>0.44</b>	0.41 [-0.03; 0.86]	<b>-0.084 [-0.164; -0.004]</b>
	$\Sigma$ PFSAs	5	37.47	-64.37	2.47	0.13		<b>-0.06 [-0.13; 0.02]</b>
	Null model	4	36.22	-64.05	2.78	0.11		<b>0.03 [-0.02; 0.07]</b>

**Table 1.** Relationships between biomarkers of energy metabolism and pollutant concentrations in female polar bears adipose tissue and plasma captured in Svalbard (2012–2013). The three most competitive models including the best predictor ( $\Delta$ AICc = 0), predictors that received strong support ( $\Delta$ AICc  $\leq$  2), conditional averaged estimates and 95% confidence intervals derived from mixed models are given. Bold values represent significant relationships, shaded rows represent the variables and relationships with  $\Delta$ AICc < 2.



levels of pollution: a maximum of two biomarkers of energy metabolism out of 16, had significantly different responses according to pollutant level (Table 2). In contrast, among bears sampled when access to sea ice was really low (April 2013), the response of seven energy metabolism biomarkers were higher in the more polluted females compared to the less polluted ones (Table 2). In addition, when we consider the biomarkers with responses close to significance ( $0.05 < p < 0.10$ ), the response to pollutants of one more biomarker presented differences between the high and low pollution groups during periods with good access to sea ice, whereas during the period with low access to sea ice, this was observed in four more biomarkers (Table 2).

## Discussion

Most biomarkers of energy metabolism in female polar bears varied in a predictable way between seasons. During their feeding state in April, the metabolic response of female polar bears resulted in higher expressions of *PPARG*, *PGC1* and *FASN* and higher concentrations of lactate and glucose compared to fasting September females. These biomarkers are involved in gluconeogenesis and FA storage, which would logically occur during a feeding state<sup>10</sup>. *SREBP1* transcript levels, *de novo* FA synthesis and triglyceride concentrations were higher whereas FA elongation index was lower in fasting than in feeding female polar bears. The concomitant action of *SREBP1* and *PPARG* lead to increased synthesis of FA which are stored as triglycerides in adipocytes and peripheral tissues<sup>7,8</sup>. Therefore, an up-regulation of *SREBP1* transcript levels, *de novo* FA synthesis and triglyceride concentrations in fasting bears is surprising. We would have expected this pattern in feeding females (April) when *PPARG* transcript levels were also upregulated and when polar bears are supposed to store lipids. Several parameters related to lipid metabolism remained unchanged between feeding and fasting females. This could occur because polar bears express some of the measured biomarkers regardless of the metabolic state and have a flexible metabolism that enables them to shift from a feeding to a fasting state regardless the season<sup>48</sup>.

The absence or occurrence of seasonal variations in biomarkers of energy metabolism could also result from pollutant exposure.  $\Sigma$ PCBs, BDE-153 and oxychlordane in polar bear adipose tissue predicted an increase of *PPARG*, *PGC1*, *FASN* and *PNPLA2* transcript levels. These four genes are involved in the accumulation of triglycerides via glucose and FFA utilization<sup>6,49,50</sup>. Accordingly, a recent *in vitro* study by Rouiti *et al.* (2016) showed that pollutants extracted from polar bear tissue increased accumulation of triglycerides in polar bear adipose tissue-derived stem cells during adipogenesis. In contrast, a synthetic mixture of pollutants containing only PCBs and organochlorine pesticides, did not affect triglyceride accumulation<sup>27</sup>. The underlying mechanisms for positive relationships between *PPARG* transcript levels and  $\Sigma$ PCBs, BDE-153 and oxychlordane exposure are likely related to other processes than direct activation of *PPARG* by the pollutants. In the *in vitro* study the most abundant POPs in polar bear adipose tissue (PCB-153 and oxychlordane) and a mixture of PCBs, chlorinated pesticides and PBDEs reflecting concentrations in polar bear adipose tissue, antagonized polar bear *PPARG*<sup>27</sup>. However, the *in vitro* study tested whether pollutants would activate a fusion protein of the yeast GAL4-DBD and the *PPARG* ligand-binding domain<sup>27</sup>, but *in vivo* *PPARG* forms a permissive heterodimer with retinoid X receptor (RXR), by which RXR ligands can also activate the *PPARG*/RXR dimer<sup>51,52</sup>.

*Trans*-nonachlor and the dioxin-like (coplanar) PCB-118 predicted an increase of *LIPE*, *ADIPOQ*, *CD36* and *FABP4* transcript levels. The resulting proteins of these four genes affect both the activity and transcript level of each other. For instance, in mice adipocytes, *FABP4* and *ADIPOQ*, respectively, increase and suppress *LIPE* catalytic activity<sup>14,53</sup>. Moreover *ADIPOQ* up-regulates *CD36* in human macrophage foam cell formation<sup>54</sup>. The observed relationships are likely to stem from effects involving the aryl hydrocarbon receptor (AhR)<sup>55</sup>. Dioxin-like PCBs (e.g. PCB-118) are *AhR* agonists<sup>56</sup> and long-term activation of AhR induces hepatic accumulation of triglycerides in transgenic mice, likely owing to the combined up-regulation of *CD36*, FA transport proteins and suppression of FA oxidation<sup>57</sup>. Similarly, *trans*-nonachlor could induce the AhR pathway up-stream or down-stream. Indeed, *trans*-nonachlor gavage in rats induced liver cell hypertrophy and increased hepatic total lipids, triglycerides and phospholipids<sup>58,59</sup>. These pathways have been extensively studied in the liver, but they likely also occur in adipose tissue, since *AhR* is expressed in white adipose tissue of several mammals<sup>60</sup>. Therefore, we may assume that PCB-118 and *trans*-nonachlor could disrupt FA transport, oxidation and insulin utilization *via* the AhR.

Finally, PFASs were related to *SREBP1* transcript levels and biomarkers of energy metabolism in plasma. This association could involve a disruption of the hypothalamic-pituitary-thyroid (HPT) axis. As previously mentioned, PFASs predicted decreased concentrations of free triiodothyronine (T3) in the present female polar bears<sup>31</sup>. In humans, and in murine and primate models, studies have reported negative associations between thyroid hormones and lipid related plasma parameters such as cholesterol, triglycerides and HDL<sup>61–63</sup>. These studies suggest that a disruption of thyroid hormones leads to disrupted levels of plasma lipids. *SREBP1* is likely an intermediate for these relationships. T3 represses *SREBP1* expression in human adipocytes, therefore lower T3 concentrations result in increased *SREBP1* expression<sup>64</sup>. Further, *SREBP1* is involved in the regulation of cholesterol homeostasis and gluconeogenesis<sup>65,66</sup>. In addition, in human dermal fibroblasts, T3 enhances the production of lactate<sup>67</sup>. Thus, the PFAS-associated decrease of thyroid hormones could account for the decrease of lactate we observed in Svalbard polar bears.

In the present study, we focused on the most significant relationships between pollutants and biomarkers of energy metabolism (i.e. highest ranked models with pollutants as predictors). Yet we cannot exclude the possibility that other pollutants not measured in this study could contribute to the disruption of energy metabolism in polar bears. For instance, suspect/non-target screening in polar bear tissue extracts indicated the presence of phthalates, nonylphenol and tonalide<sup>27</sup>, which are potential enhancers of adipogenesis<sup>68,69</sup>. Consequently, other pathways could also influence the relationships between energy metabolism and pollutants. For example, the RXR, the liver X receptor (LXR) in hepatocytes, and the pregnane X receptor (PXR) play a major role in the regulation of metabolism and are known targets for pollutants in mammals, including polar bears<sup>70–72</sup>. Furthermore, metabolic pathways can be interrupted or modified at several points and alter both expression and activity of proteins involved in lipid composition. For example, at the transcriptional level, *FASN* was positively related to  $\Sigma$ PCBs, whereas the FA *de novo* synthesis index in adipose tissue was negatively related to BDE-153. The



		Pollutant concentrations: High vs low			
		April 2012, n = 33	September 2012, n = 24	April 2013 (Stressful), n = 29	September 2013, n = 26
Transcript levels	<i>PPARG</i> <sup>a</sup>	High = Low	High = Low	High > Low*	High = Low
	<i>PGC1</i> <sup>a</sup>	High = Low	High = Low	High > Low*	High = Low
	<i>FASN</i> <sup>a</sup>	High = Low	High > Low●	High > Low●	High = Low
	<i>PNPLA2</i> <sup>a</sup>	High = Low	High = Low	High > Low*	High = Low
	<i>LIPE</i> <sup>b</sup>	High = Low	High = Low	High > Low*	High = Low
	<i>ADIPOQ</i> <sup>b</sup>	High > Low●	High = Low	High > Low●	High = Low
	<i>CD36</i> <sup>b</sup>	High = Low	High = Low	High > Low*	High = Low
	<i>FABP4</i> <sup>b</sup>	High = Low	High = Low	High = Low	High = Low
	<i>SREBP1</i> <sup>c</sup>	High > Low*	High > Low*	High = Low	High = Low
FA indexes	<i>de novo</i> synthesis <sup>c</sup>	High < Low*	High = Low	High < Low●	High < Low*
	Elongation <sup>c</sup>	High = Low	High = Low	High > Low●	High = Low
Plasma parameters	Cholesterol <sup>d</sup>	High = Low	High = Low	High > Low*	High = Low
	HDL <sup>e</sup>	High = Low	High = Low	High > Low*	High = Low
	Triglycerides <sup>c</sup>	High = Low	High > Low*	High = Low	High = Low
	Lactate <sup>e</sup>	High = Low	High = Low	High = Low	High < Low*

**Table 2.** Combined effects of sea ice and pollutants on energy metabolism. Energy metabolism response to High or Low pollutant concentrations during stressful (spring 2013) and non-stressful (spring and autumn 2012, autumn 2013) sampling periods. We used spring 2013 as a reference “stressful period”. For each group of pollutant (best predictor according to AICc), the data set was split into two classes separated by the median pollutant concentration, resulting in one high and one low polluted group for each individual or group of pollutants. “High” refers to more polluted females with pollutant and “Low” to less polluted females. Pollutants used for high - low classification: <sup>a</sup> $\Sigma_{13}$ PCBs, oxychlorane, <sup>b</sup> $\Sigma$ (PCB-118, *trans*-nonachlor), <sup>c</sup> $\Sigma_6$ PFCA, <sup>d</sup> $\Sigma_2$ PFSA, <sup>e</sup>BDE-153. \*p < 0.05, ●p < 0.10.

two seasons, spring (April) and autumn (September) 2012 and 2013. The bears were immobilized by remote injection and blood samples and adipose tissue biopsies were collected from each bear.

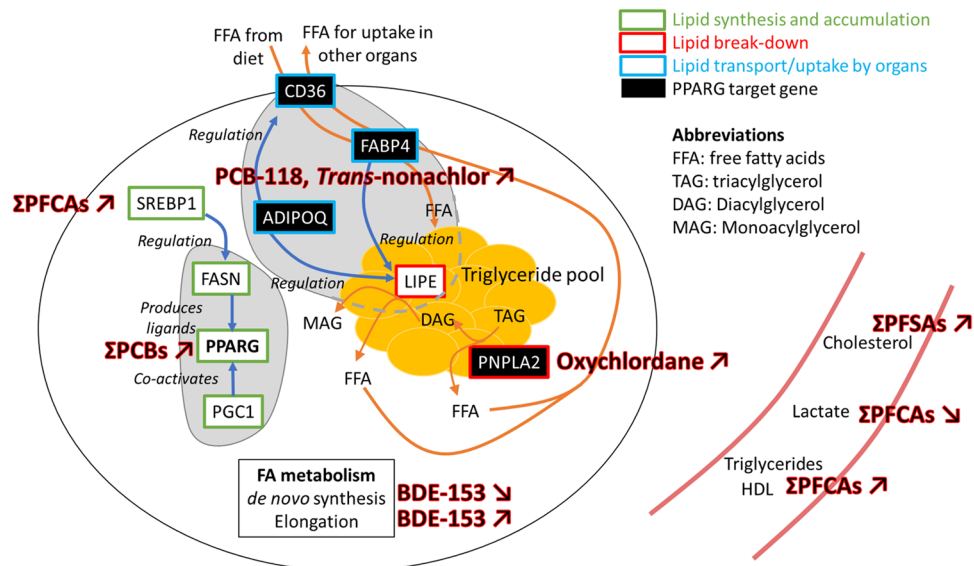
Fieldwork was carried out in accordance with relevant guidelines and regulations from the Governor of Svalbard and was approved by the Norwegian national animal research authority (FOTS).

**Gene description and transcript level analysis by relative quantitative real-time PCR (qPCR).** The studied genes in adipose tissue included *PPARG*, *PGC1*, *FASN*, *PNPLA2*, *LIPE*, *ADIPOQ*, *CD36*, *FABP4* and *SREBP1*. Total RNA was extracted from adipose tissue samples in the 112 polar bear samples using RNeasy Lipid Tissue Mini kit (QIAGEN, Oslo Norway) and cDNA produced from 1  $\mu$ g RNA using qScript cDNA Synthesis Kit (Quanta Biosciences, Massachusetts, US). Quantitative real-time PCR was performed using SYBR Green I Master on a LightCycler<sup>®</sup> 480 (Roche, Basel, Switzerland). Detailed information on cycling conditions, establishment of standard curves, amplification efficiency and specificity is found in the supporting information (Table S2, Figure S1). Specific amplification of target and reference genes was confirmed by evaluation of melting curves and by agarose gel electrophoresis (Supporting information). Gene transcript levels were analyzed at the University of Bergen (UiB, Bergen, Norway).

**Determination of FAs from adipose tissue and FA indexes.** Proportional mass data for 33 FAs were derived from 83 of the 112 polar bear samples, following methods described in the supporting information. We calculated two ratios between individual FAs as proxies of 1) FA *de novo* synthesis, which provides information on the conversion of excess carbohydrates to FAs and triacylglycerol<sup>89</sup> and 2) FA elongation which provides information on the elongation of saturated and monounsaturated FA into very long chain FAs<sup>90</sup>. The ratios between individual FAs were calculated as follows: FA *de novo* synthesis (16:0/18:2n - 6)<sup>89</sup> and FA elongation index ((18:0 + 18:1n - 9)/16:0)<sup>73,90</sup>. FA analysis was conducted at York University (Toronto, ON, Canada) and Dalhousie University (Halifax, NS, Canada).

**Lipid parameters determination in plasma.** Plasma cholesterol, triglyceride and HDL concentrations (n = 111, mmol/L) were determined using a “dry” clinical-chemical analyzer, Reflotron<sup>®</sup> (Model IV, Boehringer-Mannheim GmbH, Mannheim, Germany) at the Norwegian University of Science and Technology (NTNU, Trondheim, Norway). Methods are detailed in the supporting information.

**Metabolome and lipidome.** In plasma samples, polar metabolites (n = 111) and lipids (n = 101), were separated as detailed in the supporting information. The detected metabolite features, including ion source fragments, adducts and isotopic ions, are defined by a unique combination of m/z and retention time value<sup>91</sup>. The metabolome (low molecular weight polar metabolites) was characterized by nuclear magnetic resonance spectroscopy. The lipidome was characterized by fingerprints obtained from flow injection time-of-flight mass spectrometry with electrospray ionization. The most important polar metabolites that were affected by environmental



**Figure 3.** Schematized functions of the genes of interest and summary of the relationships between pollutants and biomarkers of energy metabolism in (A) a white adipocyte and (B) plasma. For a detailed description of lipid metabolism in white adipocyte see Sethi and Vidal-Puig (2007)<sup>106</sup>.

and/or physiological factors were quantified by integration against trimethyl-silyl-propionate in MestreNova 8.1 (MestreLab Research S.L) and lipids were quantified by their exact mass (m/z) via Fourier transform ion cyclotron resonance. Metabolome and lipidome were measured at SINTEF (Trondheim, Norway).

**Pollutant determination in plasma and fat.** OHCs were determined at the Norwegian University of Life Science (NMBU, Oslo, Norway). Concentrations of lipophilic pollutants (ng/g wet weight) such as PCBs, OCPs and BFRs were determined in adipose tissue (n = 111), whereas non-lipophilic pollutants such as perfluoroalkyl substances (PFASs) were determined in plasma (n = 112). Methods and limits of detection (LOD) have been reported elsewhere<sup>39,92</sup>. The compounds used for statistical analyses were those that were above the LOD in more than 90% of the samples. In adipose tissue, this was the case for: 14 PCBs (CB-99, -118, -137, -138, -153, -156, -157, -170, -180, -183, -189, -194, -206 and -209), oxychlordane, *trans*-nonachlor, HCB, and four BFRs (BDE-47, -100, -153, hexabromocyclododecane). In plasma, the concentrations of the following compounds were detected in >90% of the samples: six PFCAs with 8 to 13 carbons (perfluorooctanoate, perfluorononanoate, perfluorodecanoate, perfluoroundecanoate, perfluorododecanoate and perfluorotridecanoate) and two PFASs with 6 and 8 carbons (perfluorohexane sulfonate and perfluorooctane sulfonate). If a compound had one or more value below LOD we generated random numbers (“*runif*” function in the software R-3.2.5) ranging between the LOD value and ½ LOD. The pollutant levels and variations according to season, body condition diet and breeding status have already been discussed in previous studies<sup>39,92</sup>.

**Combined effects of pollutants and sea ice conditions.** During the four sampling periods of this study (April 2012, September 2012, April 2013 and September 2013), April 2013 was considered as a “stressful period” due to the severely poor sea ice conditions during the previous winter<sup>39</sup>. During winter/spring 2013, Svalbard was mostly ice-free and sea ice concentrations rarely exceeded 12.5%, as opposed to during winter/spring 2012 when sea ice concentrations varied from 25% to more than 50%<sup>93</sup>. Furthermore, the abundance of preferred sea ice habitat available to polar bears was low during the months preceding the sampling period in April 2013 compared to the months preceding the other sampling periods<sup>92</sup>. As compared to the other 3 capture periods, in April 2013 a larger proportion of female polar bears were under nutritional stress as suggested by lower body condition, larger proportion of fasting females (higher urea to creatinine ratio) and higher  $\delta^{15}\text{N}$  values, suggesting that females were catabolizing their protein pool, thus under nutritional stress<sup>39,92</sup>.

**Data availability and statistical analyses.** All data are made available on the Norwegian Polar Institute data repository (data.npolar.no). Statistical analyses were performed using R 3-2.5<sup>94</sup>. First, we investigated the effects of metabolic state (i.e. if the bears were fasting versus feeding) on biomarkers of energy metabolism. To do so, we considered season as a proxy of metabolic state and assumed that most females captured in April were feeding whereas most females captured in September were fasting<sup>92</sup>. We then tested the effect of season on biomarkers of energy metabolism by using GLMM (R package *nlme* version 3.1–121<sup>95</sup>) with female identity as a random factor.

The best predictors of the concentrations of lipophilic and proteinophilic pollutants in the polar bears used in this study were previously determined to be body condition and diet, respectively, which are both related to season<sup>31,39,92,96</sup>. We tested the effects of age, body condition index (BCI), body mass and diet proxies (nitrogen and carbon stable isotope values in red blood cells) on energy metabolism biomarkers (Table S3). All energy



biomarkers were either predicted by BCI, body mass or diet proxies (Table S3) and as previously mentioned BCI, body mass and diet proxies are all confounded with season<sup>31,39,92,96</sup>. To remove the possible confounding effect of season and test the effect of pollutants on metabolism biomarkers, we used GLMMs with pollutant concentrations as fixed factors, and, female identity and season as random factors. Prior to this, we performed a RDA<sup>97,98</sup> to visualize the potential relationships between pollutants and biomarkers, and the pollutants were then grouped according to their proximity in the RDA loading plot. Then, for each response variable we built ten candidate models that included all the pollutants, individually or summed and the null model. We used conditional model averaging to make inferences from all the GLMMs. To rank the models we used an information-theoretic approach<sup>99</sup> based on Akaike's information criterion corrected for small sample size (AICc, R package *MuMIn*)<sup>100</sup>. The number of parameters (K), the difference in AICc values between the “best” model and the model at hand ( $\Delta\text{AICc}$ ) and a normalized weight of evidence in favor of the specific model, relative to the whole set of candidate models, derived by  $e^{(-0.5(\Delta\text{AICc}))}$  (AICc weights) were calculated. We calculated averaged estimates for all predictor variables in the candidate model list, weighted using the AICc weights<sup>99,101</sup>. We obtained conditional parameter-averaged estimates ( $\beta$ ) and 95% confidence intervals (CIs) for the predictors (individual or summed pollutants) included in the most competitive models (Table 1). We used 95% CI of the model averaged estimates to determine if parameters were significantly different from 0 at the 5% level. To determine if season could predict metabolome and lipidome raw data, we used PLS and MANOVA. Further, we studied relationships between the lipidome and pollutant concentrations. Using the mixOmics library in R<sup>102</sup> a sparse PLS<sup>103,104</sup> with multi-Y response was conducted with pollutant concentrations as response variables and the lipidome as predictor variables. Keeping 29 response and predictor variables, correlations between lipids and pollutants were observed.

Finally, to test for possible combined effects of sea ice conditions and internal pollutant levels on energy metabolism, we checked if the response of biomarkers of energy metabolism to pollutant concentrations differed between sampling periods qualified as “normal” such as April 2012, September 2012 and September 2013 or “stressful” (April 2013). We selected the pollutants that mostly influenced the biomarkers of energy metabolism (see Table 1). For each group of pollutant (best predictor according to AICc), the data set was split into two classes separated by the median pollutant concentration, resulting in one high and one low polluted group for each individual or group of pollutants. Due to variation in the internal concentrations of various pollutants in the bears, the individual bears were not necessarily the same in each group, according to the pollutant considered. Then, we compared the responses (concentrations, transcript levels) of biomarkers of energy metabolism between more and less polluted females for each period (spring 2012, autumn 2012, spring 2013 and autumn 2013). We used least square means (LSMs) comparisons to check whether the metabolic response of female polar bears from the more or less polluted groups differed for each sampling period. In unbalanced factorial experiments, LSMs for each factor mimic the main-effects means, but are adjusted for imbalance<sup>105</sup>.

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### Author Contributions

Conception and design of the work: H.R., J.A. Data collection: H.R., J.A., S.B. Laboratory determination on lipid biomarkers: R.L.L., S.B., V.T., G.W.T., A.B., T.R.S. Analysed and interpreted the data, drafted the article: S.T. Critical revision of the article: H.R., S.B., B.M.J., G.W.T., A.G., R.L.L., A.P., V.T. Final approval of the version to be published: all the authors.

### Additional Information

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