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The brown bear as a translational model for sedentary lifestyle-related diseases

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Abstract. Fröbert O, Frøbert AM, Kindberg J, Arnemo JM, Overgaard MT (Örebro University, Örebro, Sweden; Aalborg University, Aalborg, Denmark; Swedish University of Agricultural Sciences, Umeå, Sweden; Norwegian Institute for Nature Research, Trondheim; Inland Norway University of Applied Sciences, Koppang, Norway). The brown bear as a translational model for sedentary lifestyle-related diseases (Review-Symposium). *J Intern Med* 2020; **287**: 263–270.

Sedentary lifestyle accelerates biological ageing, is a major risk factor for developing metabolic syndrome and is associated with cardiovascular disease, diabetes mellitus, kidney failure, sarcopenia and osteoporosis. In contrast to the linear path to worsening health in humans with metabolic syndrome, brown bears have developed a circular metabolic plasticity enabling these animals to tolerate obesity and a 'sedentary lifestyle' during hibernation and exit the den metabolically healthy in spring. Bears are close to humans physiology wise, much closer than rodents, the preferred experimental animals in medical research, and may better serve as translational model to develop treatments for lifestyle-related diseases. In this review, aspects of brown bear hibernation survival strategies are outlined and conceivable experimental strategies to learn from bears are described.

Keywords: brown bear, hibernation, metabolic syndrome, translational research.

Background

Brown bears (*Ursus arctos*) and black bears (*Ursus americanus*) hibernate physically inactive for up to 6 months without eating, drinking, or defecating, and with no or intermittent urination. Upon exiting, the den in spring, bears are free from cardiovascular disease, kidney failure, sarcopenia, osteoporosis and other deleterious conditions. The contrast to physically inactive humans could not be greater.

Sedentary lifestyle in an unadapted species

Over a short time span in evolutionary terms, humans have gone from being nomadic hunter–gatherers to a settled, agrarian-based way of living in communities followed by industrialisation where the intent of physical activity was to produce needed materials. Ninety-nine per cent of our evolutionary history was formed as hunter–gatherers following a highly mobile lifestyle, and our gene pool was shaped by natural selection towards an optimal adaptation to these environments [1]. Many now live in post-industrial societies where the majority is not regularly physically active and a large number of people are not active at all [2]. Easy access to high-calorie foods of poor nutritional value rich in refined sugars and unsaturated fat has contributed to a pandemic of lifestyle-related noncommunicable diseases.

It is safe to say that humans are not well suited for sedentary behaviour. Physical inactivity speeds biological ageing and is an actual cause of over 35 chronic diseases/conditions, whose outcome decreases lifespan [3]. For years, scientific literature and health guidelines have universally recommended daily physical activity and warned against overeating but global effects on sedentary lifestyle-related diseases have been paltry. Humans suffering from obesity, metabolic syndrome or type II

diabetes mellitus are on a trajectory to worsening health. No interventions, whether medication [4], education [5] exercise [6] or gastric bypass surgery [7], can reverse the process but merely act as moderators. The majority of research on animal models used to map metabolic disorders and discover new treatments are rodents, which share these apparent pre-programmed detrimental features with humans. Animals evolutionarily adapted to periodic obesity and long periods of immobilisation may hold keys to prevention and treatment strategies for humans.

Rodents – inadequate models for lifestyle-related diseases

Currently, rodents are by far the most frequently used animal model in metabolic syndrome, diabetes, obesity and cardiovascular research, and the literature is overwhelmed with reports on genes having a role in disease development in rats and mice. Although these models have provided many useful insights in basic metabolism, a number of shortcomings exist. Experimental mice and rats are inbred, hypertensive, glucose-intolerant, prone to cancer and kidney failure, and have a shorter lifespan than animals living in the wild [8]. Most often, these animals are kept at room temperature, well below their thermoneutral zone (30–32°C for mice) which, in combination with light and absence of hiding space, cause constant physiological stress [9]. Focusing on a few animal species confines the providence to those particular organisms [10], partly explaining why translation of findings from laboratory animal research to humans often fails [11, 12]. A more informative approach would be to study free-ranging animals with alternative evolutionary solutions of immobility and handling fluctuating access to food. By investigating such animals, we might identify novel factors or pathways that can leverage our understanding of human lifestyle-related diseases.

Bear hibernation physiology

Brown (and black) bears are unique amongst hibernating animals. Bears endure being physically inactive inside their winter dens for half a year whilst preserving organ function, avoiding thromboembolism, heart failure, disuse osteoporosis and severe sarcopenia (Fig. 1). Contrary to most other hibernators, brown bears are shallow hibernators with a certain amount of alertness during the entire hibernation period and only a slight decrease in body temperature to approximately 33–34°C [13]. Regardless of not hibernating at severe hypothermia bears lower metabolic rate to 25% of active state [14] under low-flow hemodynamics with heart rates as low as 10 beats per minute and respiratory rates of 1–2 per minute [15, 16]. Despite a huge number of studies on hibernation physiology in several species, positive selection signatures in the coding sequences of genes have not been found [17]. The primary drivers of the hibernation phenotype should therefore be sought in gene regulation but such drivers remain elusive [18].

Metabolism

The mechanisms involved in metabolic depression in bears during hibernation are not well understood but changes in Sulphate metabolism may play a key role and may also be important for antioxidant defence [19]. Putative hibernation triggers like 5′-adenosine monophosphate, thyronamines, 2′-deoxyglucose (2-DG), and delta-opioids seem to be linked to hypothermia, a key feature of small animal hibernation [18], and no single substance or combination of substances have been documented to induce or sustain hibernation in bears.

In late summer and fall, brown bears exhibit hyperphagia and gain up to 30% in body mass compared to spring [20] whilst, in contrast to obese humans, preserving insulin sensitivity [21]. In order to facilitate weight gain of this magnitude whilst active and secure fuels during starvation in hibernation a dramatic metabolic switch is required. Studies on transcriptional regulation of metabolism-related genes in white adipose tissue and skeletal muscle in black bears demonstrate upregulation of lipogenesis-related genes in summer and downregulation of genes involved in glycolysis and upregulation of lipolysis genes during hibernation [22]. Potent upregulation of inhibitors of lipolysis (CIDE-C and G0S2) in adipose tissue is seen during summer whilst in winter breaks on lipolysis are almost absent and levels of the prolipolytic cofactor CGI-58 are elevated [23]. Some of the hibernation-induced metabolic changes might be driven by reduced diversity of gut microbiota including reduced levels of Firmicutes and Actinobacteria and increased levels of Bacteroidetes. Transplantation of bear microbiota from summer and winter to germ-free mice demonstrates that summer microbiota promote adiposity without impairing glucose tolerance [24].

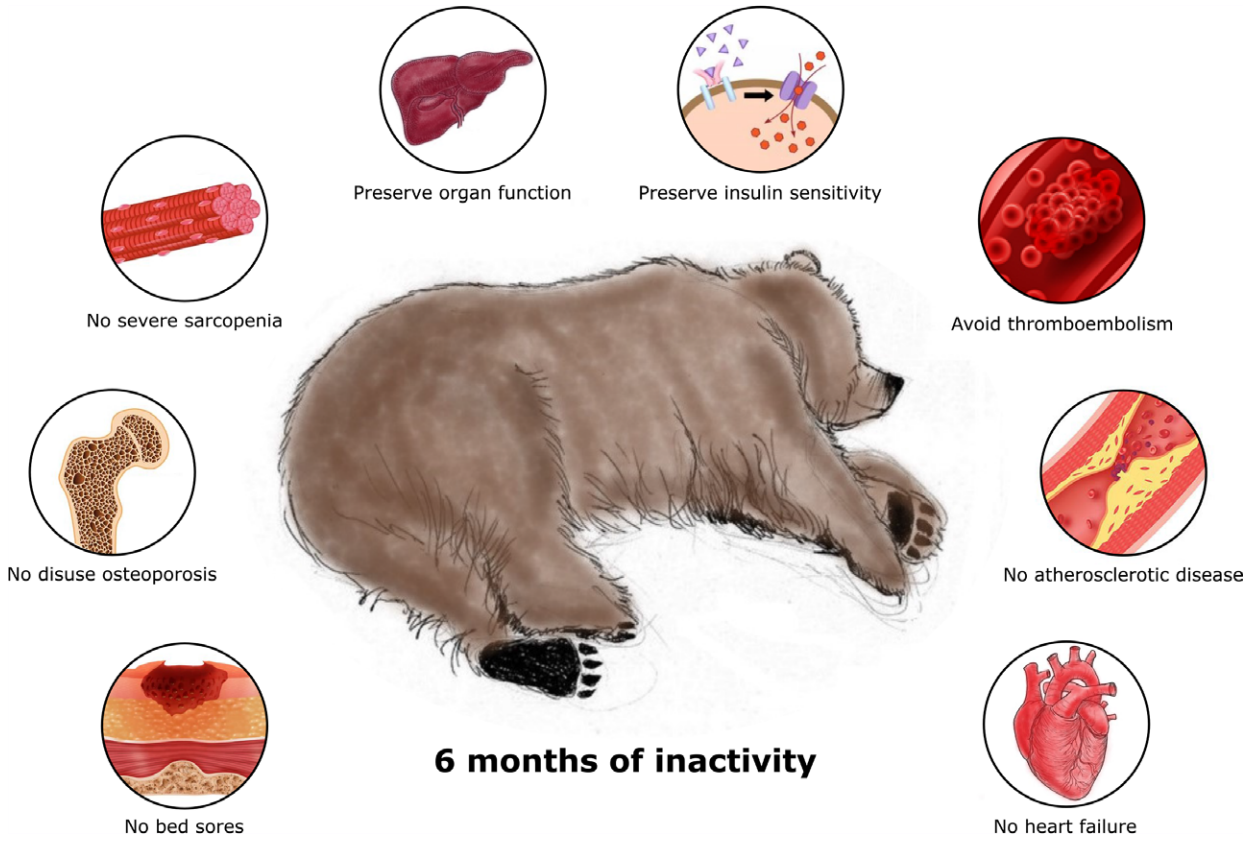

Fig. 1 *Brown bears hibernate physically inactive for 6 months each year. When exiting the den in spring, bears are free from cardiovascular disease, kidney failure, sarcopenia and osteoporosis. Brown bear physiology may hold keys to prevent diseases in humans related to physical inactivity.*

Circulation and coagulation

The circulation is in a low-flow state during brown bear hibernation. Heart rate goes down to 10 beats per minute [25], asystoles are frequent, and cardiac output is reduced to 25% compared to active state [26] but bears have no signs of heart failure. Low flow is associated with thrombus formation in humans [27] but not in bears. The ability to avoid blood clots seems, at least partly, to be explained by spontaneously reduced platelet aggregation in bears during hibernation [28, 29] but also by downregulation of key coagulation factors [30, 31]. However, levels of the ultimate clotting effector proteins F10 and F2, and those of the fibrinogen substrate subunits FGA and FGG increase in winter in brown bears and indicate some degree of preserved coagulation in order to avoid bleeding. Downregulation of most coagulation pathway proteins and upregulation of only a few key proteins mimics to some extent regression to a primitive species coagulation system in bears during hibernation [32].

Puzzlingly, bears also have high cholesterols, a risk factor for atherosclerosis in humans. But despite this and annual periods of obesity and inactivity, bears do not develop atherosclerotic disease [33].

Whilst hibernating, bears breathe 1–2 times per minute and O_2 consumption rate is downregulated by 75% [14]. Oxygen affinity in bears is associated with a decrease in the red cell haemoglobin cofactor 2,3-diphosphoglycerate (DPG) during hibernation to approximately half of the summer value causing a left shift in the Hb-O_2 equilibrium curve to adapt to a decreased O_2 supply to tissues during hibernation ensuring oxygenation of vital tissues like the brain and heart [34].

Skeletal muscle

Atrophy of skeletal muscle during disuse is seen in humans during immobilisation, bed rest, spaceflight, denervation, chronic kidney failure, cancer, and ageing and sarcopenia is associated with disability, poor quality of life, and increased risk of death [35]. In contrast, muscle mass and strength are well preserved in hibernating bears [36, 37]. A muscle biopsy study in black bears demonstrated increased protein synthesis in summer, which was greater than breakdown indicating muscle protein accumulation. In winter, both protein synthesis and breakdown were lower compared to summer with no difference early and late during hibernation, indicating protein balance. Suggested underlying mechanisms involve downregulation of myostatin expression to counteract protein degradation and a shift in muscle phenotype towards slow-oxidative fibre and mitochondrial biogenesis during hibernation [38]. In a comparative study of gene expression in hindlimb muscles from hibernating and summer-active black bears and arctic ground squirrels, a similar proportion of genes was over-expressed during hibernation in both species leading the authors to conclude that a common transcriptional increase in transcriptional levels of anabolic genes involved in protein biosynthesis is largely independent of body temperature (near-normal in bears, close to 0°C in ground squirrels) [39]. Interestingly, in a study exposing human skeletal muscle cells to brown bear summer and winter serum, protein turnover of myotubes was reduced when incubated with winter serum, with a dramatic inhibition of proteolysis involving both proteasomal and lysosomal systems and resulting in an increase in muscle cell protein content [40].

Bone

Despite being physically inactive during hibernation, no signs of disuse osteoporosis have been demonstrated in bone structure in bears [41–43] and cortical porosity does not increase, which is in contrast to what is observed in immobilised humans [44]. In a study on brown bears, we found that vitamin D in the form of 25-hydroxy-ergocalciferol was higher in winter than in summer whilst total serum calcium and parathyroid hormone levels did not differ. Osteocalcin levels were higher in summer than winter whereas other markers of bone turnover (ICTP and CTX-I) were unchanged [45]. A later study on black bears documented that cocaine and amphetamine regulated transcript (CART), a hormone known to reduce bone resorption by inhibiting genesis of osteoclasts, was 15-fold higher during hibernation [46]. Another piece to the puzzle may come from stem cells. Bone regeneration is a complex process involving mesenchymal stem cell invasion, chondrogenesis, osteogenesis and angiogenesis. Adipose tissue-derived stem cells from brown bears spontaneously form bone-like nodules surrounded by cartilaginous deposits, suggesting differentiation into osteogenic and chondrogenic lineages [47]. Spontaneous stem cell differentiation is unique and has only been described in a few other cell types across species. The overall picture of markers of bone remodelling in bears leaves an impression that the skeleton appears to perceive that it was loaded when it was actually unloaded during hibernation [48].

A strategy to translate from bears to humans

The brown bear can be used as a translational animal model to explore metabolic survival strategies for insights in and future treatments of metabolic disorders. The bear is obese when entering hibernation in the fall, and for six months, the bear is immobile, burns almost exclusively fat and displays only a slight reduction in lean body mass. In contrast to the linear path to worsening health in humans with metabolic syndrome, bears have developed a circular metabolic plasticity enabling these animals to tolerate obesity and a 'sedentary lifestyle' and exit the den metabolically healthy in spring. Bears are phylogenetically relatively close to humans, and the ability to hibernate is not genetically defined but likely confined to differently expressed proteins [13] which increases the likelihood that features of brown bears hibernation can inform treatment strategies for humans.

To elucidate the adaptive molecular strategies of bears, The Scandinavian Brown Bear Research Project (http://bearproject.info/) collects samples for research purposes from free-ranging brown bears (*Ursus arctos*) located in Sweden. Blood samples and tissue biopsies are collected during hibernation in winter and from the same bears during the active summer period [49]. Comparison of the molecular conditions of summer and winter samples can reveal information of the local and systemic regulations involved in the extraordinary metabolic transformation to hibernation.

As proteins are involved in all regulatory pathways, proteome analyses and study of the dynamics in protein expression can provide information of pathways involved in the adaptions for hibernation. Proteomes of blood, organs and tissues can be characterised by mass spectrometry (MS), which identifies proteins and determines relative protein quantities by matching the mass of peptide fragments to predicted peptide sequences from databases.

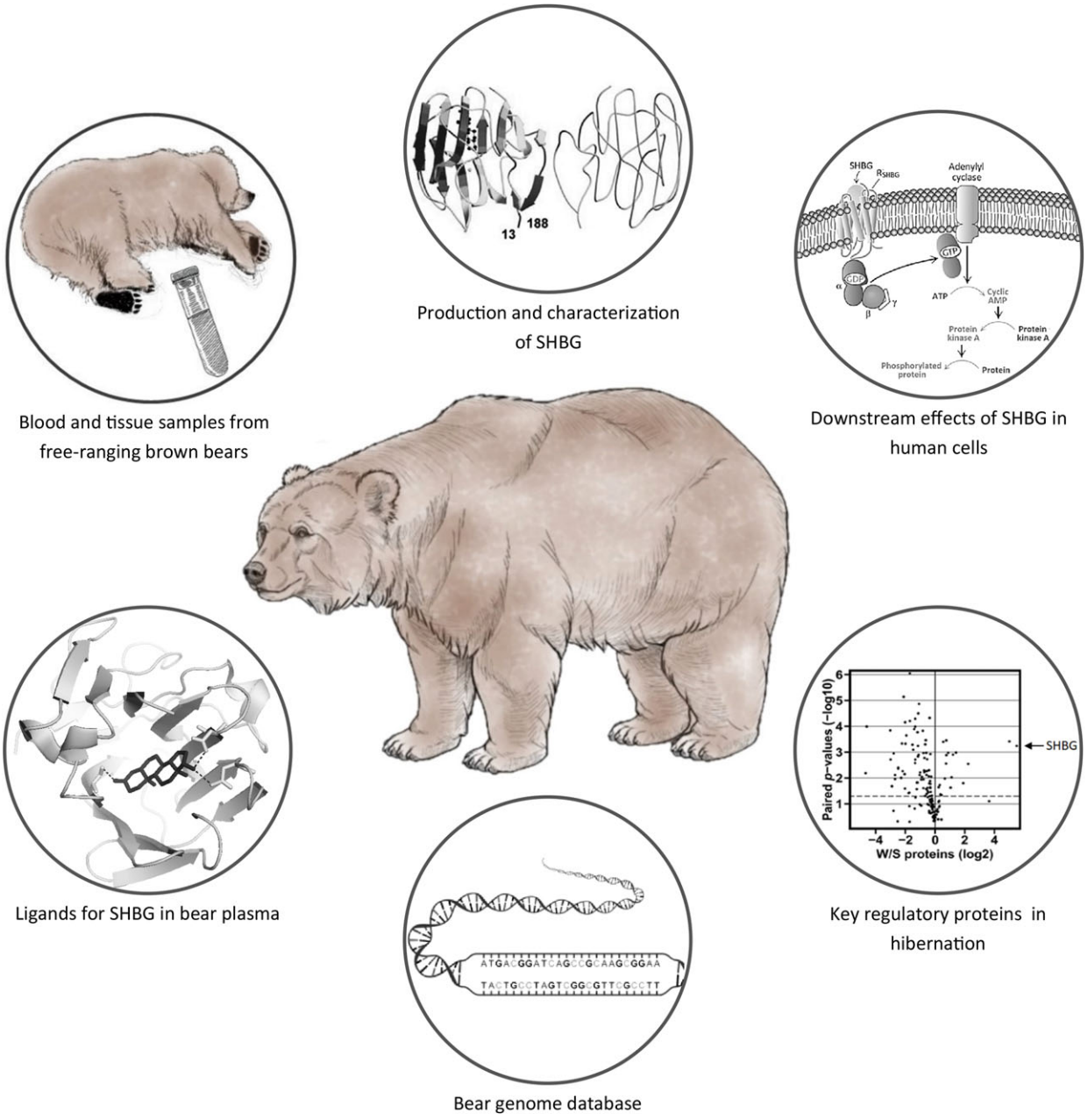

Fig. 2 *Sex hormone-binding globulin (SHBG) is increased 45-fold during hibernation in brown bears and could be the primary endocrine mediator of hibernation physiology in this animal. The figure illustrates putative steps in defining the role of SHBG.*

The differences between the summer and winter brown bear plasma proteome have been characterised by Welinder et al. [30], providing information of the biochemical adaptions for hibernation, as the bloodstream has primary functions in signalling. A number of adaptations specific to hibernation were discovered, including energy conservation by decreased levels of the majority of plasma proteins combined with maintained or moderately increased levels of a few key plasma proteins performing crucial functions in hibernation. Amongst the upregulated plasma proteins, one protein, sex hormone-binding globulin (SHBG), increased 45-fold during the hibernation period, suggesting a significant but unknown role in maintaining hibernation physiology. Our hypothesis is that the elevated level of SHBG could be the primary endocrine mediator of the remarkable physiological adaptions for hibernation observed in bears, as the fact that the entire body is affected by adaptions to hibernation is consistent with a systemic regulator. SHBG might serve its function by eliminating all free steroid function or by interactions with an uncharacterised membrane-bound SHBG receptor [50]. A function of SHBG during hibernation that is concentration as opposed to structure dependent would support a therapeutic potential of increased human SHBG levels (Fig. 2).

High circulating SHBG levels in humans appear to correlate with good health, including protection against metabolic syndrome [51], type 2 diabetes [52], obesity [53], and increased insulin sensitivity [54, 55] as well as being associated with lower risk of cardiovascular disease, including reduced blood pressure, cholesterol and triglycerides along with higher HDL-cholesterol levels [56, 57]. SHBG may also contribute to muscle maintenance [58].

Access to accurate and complete brown bear genome and transcriptome databases is a prerequisite for identifying peptides and proteins through proteomics, and can be established by sequencing of genomic DNA and mRNA isolated from brown bear tissue samples.

The protein identification by Welinder et al. [30] was based on mapping to a predicted protein database derived from the genome sequence of polar bear (*Ursus maritimus*) annotated by BlastP against the nonredundant human NCBI Reference Sequence Database, which was the only database available at the time. Since then, the genome sequence of the grizzly bear (*Ursus arctos horribilis*) has been published, which has a higher sequence identity to the genome of the Scandinavian Brown Bear (*Ursus arctos arctos*) [59]. However, only a predicted transcriptome database, based on RefSeq mRNA alignments, is available, which might comprise incorrectly predicted splice variants. Reanalysis of the MS data by Welinder et al. using a more accurate database will significantly increase the power of the proteomics analysis and likely lead to identification of additional regulatory molecules of hibernation.

A strategy to circumvent the need for an accurate and complete transcriptome database in proteomics is to use RNA-Seq to characterise the transcriptome [60]. RNA-seq likely leads to better transcript identification, as single nucleotide variants, not present in the applied database, is not missed as they are in proteomics. However, quantities of transcripts determined by RNA-seq might not reflect the true protein quantities.

Adipose tissue-derived stem cells have a capacity for self-renewal and multipotential differentiation and could be involved in preventive mechanisms of tissue atrophy or organ damage [47, 61], which do not occur in hibernating brown bears. Study and characterisation of ursine progenitor cells could lead to identification of human progenitor cells of similar potential.

Conclusion

During evolution, brown bears have adapted to a lifestyle comprising hyperphagia and obesity in the fall and immobilisation during up to half a year of hibernation in winter. Bears have developed a circular metabolic plasticity enabling these animals to avoid noncommunicable diseases such as metabolic syndrome, diabetes and cardiovascular disease, and they are not prone to osteoporosis or sarcopenia. It is likely that bears hold keys to gain insight into and perhaps treat lifestyle-related diseases in humans. Because hibernation is not genetically defined but likely confined to differently expressed proteins, a way forward is to screen for up- and downregulated peptides and proteins to identify biomarkers responsible for inducing and sustaining a healthy physiology during hibernation.

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Conflict of interest statement

No Conflict of interest was declared.

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