

Betaine reduces the expression of inflammatory adipokines caused by hypoxia in human adipocytes

K. Olli*, S. Lahtinen, N. Rautonen and K. Tiihonen

DuPont Nutrition and Health, Kantvik Active Nutrition, Sokeritehtaantie 20, FIN-02460 Kantvik, Finland

(Submitted 3 October 2011 – Final revision received 11 January 2012 – Accepted 26 January 2012 – First published online 19 March 2012)

Abstract

Obesity is characterised by a state of chronic low-grade inflammation and the elevated circulating and tissue levels of inflammatory markers, including inflammation-related adipokines, released from white adipose tissue. The expression and release of these adipokines generally rises as the adipose tissue expands and hypoxic conditions start to develop within the tissue. Here, the effect of betaine, a trimethylglycine having a biological role as an osmolyte and a methyl donor, on the expression of inflammation-related markers was tested in human adipocytes under hypoxia. Differentiated adipocytes were cultivated under low (1%) oxygen tension for 8–20 h. The expression of different adipokines, including IL-6, leptin, PPAR γ , TNF- α and adiponectin, was measured by quantitative PCR by determining the relative mRNA level from the adipocytes. Hypoxia, in general, led to a decrease in the expression of PPAR γ mRNA in human adipocytes, whereas the expression levels of leptin and IL-6 mRNA were substantially increased by hypoxia. The cultivation of adipocytes under hypoxia also led to a reduction in the expression of TNF- α mRNA. The results showed that hypoxia increased the relative quantification of leptin gene transcription, and that betaine (250 μ mol/l) reduced this effect, caused by low oxygen conditions. Under hypoxia, betaine also reduced the mRNA level of the pro-inflammatory markers IL-6 and TNF- α . These results demonstrate that the extensive changes in the expression of inflammation-related adipokines in human adipocytes caused by hypoxia can be diminished by the presence of physiologically relevant concentrations of betaine.

Key words: Adipocytes: Betaine: Hypoxia: Inflammation: Obesity

White adipose tissue is an important endocrine organ, secreting several key hormones which are part of the large group of cellular signalling factors termed adipokines. Many of these adipokines are linked to immunity and inflammatory responses. These include important cytokines and chemokines, such as IL-6, IL-8, IL-10, leptin, monocyte chemoattractant protein-1, TNF- α , transforming growth factor- β and plasminogen activator inhibitor-1. The production of these signalling factors by adipose tissue is increased in obesity. During the expansion of adipose tissue mass in obesity, there is a major inflammatory response in the tissue, with increased expression and release of adipokines related to inflammation⁽¹⁾. Hypoxia has been demonstrated to occur in fat depots inside the expanding adipose tissue mass in both obese animals^(2,3) and obese human subjects⁽⁴⁾. As the adipose tissue expands, the enlarged adipocytes become more and more distant from the vasculature. Partly as a response to this relative hypoxia, the production of pro-inflammatory adipokines is increased in the adipose tissue mass^(5–7). The elevated circulating

and tissue levels of several acute-phase proteins and pro-inflammatory cytokines contribute to the development of chronic low-grade inflammation in the adipose tissue^(6,8,9).

In the obese state as well as in hypoxia, the expression and secretion of several factors, such as IL-6, leptin and adiponectin, are disturbed. With the exception of adiponectin, which is decreased, the level of these circulating factors is elevated in obesity⁽⁸⁾. Since most of these factors are pro-inflammatory, obesity can be described as an inflammatory condition and inflammation is linked to hypoxic conditions that have developed in the adipose tissue⁽⁶⁾. Adipose tissue also plays an important part in glucose homeostasis. Hypoxia stimulates glucose transport by adipocytes and hence may alter normal cell function within the adipose tissue^(5,10). The inflammatory state in the adipose tissue of the obese can be causally linked to insulin resistance and the metabolic syndrome⁽¹⁰⁾. An excess of adipose tissue mass increases the risk for a number of conditions, including coronary artery disease, hypertension, dyslipidaemia, type 2 diabetes and cancer⁽¹¹⁾.

Abbreviations: FD, fold difference; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HIF-1 α , hypoxia-inducible factor-1, α subunit.

* **Corresponding author:** K. Olli, email kaisa.ollid@danisco.com

Betaine, also known as trimethylglycine or glycinebetaine, is found in every kingdom of life. The main dietary sources of betaine are typically grain products^(12–14). It functions as an organic osmolyte, protecting cells under hyperosmotic stress, and acts as a methyl donor in many biochemical pathways. In humans, betaine insufficiency is associated with several metabolic disorders and CVD^(15,16). Betaine has been extensively used as a feed ingredient in livestock production due to its positive impact on both animal performance and carcass quality⁽¹⁷⁾. However, the efficacy of betaine in reducing carcass fat in pigs seems to depend on dietary energy content⁽¹⁸⁾. In high-fat diet animal models, betaine has been shown to improve hepatic and adipose tissue function by correcting insulin resistance and abnormal adipokine production⁽¹⁹⁾. Methionine/homocysteine metabolism and improved insulin signalling pathways have been proposed as the mechanisms underlying the beneficial effect of betaine on adipose tissue^(19,20).

In the present study, we examined the relative expression and secretion of inflammation-related adipocyte-derived factors in differentiated human omental adipocytes stressed with 1% hypoxia. This condition is close to the adipose tissue oxygen level detected in obese mice⁽³⁾. The present study indicates that the hypoxia-stress adipocyte model can be used in examining the effects of different circulating nutrients on adipocyte function. We show that betaine may reduce the inflammatory status of adipocytes caused by hypoxic conditions.

Experimental methods

Cell culture

Human visceral preadipocytes (OP-F-2; Zen-Bio, Inc.) were derived from adipose tissue in the omentum of an African-American female subject (age 40 years, BMI 26.1 kg/m², non-smoker and non-diabetic). Written informed consent was obtained from the donor. Fibroblastic omental preadipocytes were plated at a density of 40 000 cells/cm², and differentiated into mature adipocytes according to the manufacturer's instructions using the cell-culture media OM-PM, OM-DM and OM-AM (Zen-Bio, Inc.). At day 14 post-induction, approximately 80–90% of the cells were differentiated, as confirmed by microscopic evaluation (data not shown). Adipocytes were treated, post-differentiation, with and without betaine, exposed to hypoxic conditions (1% O₂, 94% N₂ and 5% CO₂) and maintained in a CO₂ incubator (HeraCell 150i; Thermo Fischer Scientific). The cells were stressed with hypoxia for 8 h (short-term hypoxia induction) to 20 h (long-term hypoxia induction). The normoxic control cells were cultivated in a standard O₂ atmosphere (21% O₂ and 5% CO₂). Each treatment was administered in six separate wells, i.e. each treatment was run in six replicates. Before RNA extraction, two replicate samples were pooled, leaving triplicate treatments for gene expression analysis.

Anhydrous betaine (Betafin[®] BP20; Danisco Finnfeeds) was used in three concentrations, 50, 250 and 500 μmol/l, which correspond to the average concentration of betaine in

human plasma and peak concentrations in the serum after single doses of 1 and 3 g of betaine, respectively⁽²¹⁾. Betaine was administered in the media either simultaneously or 16 h before hypoxia induction.

RNA extraction, complementary DNA synthesis and real-time PCR

Total RNA was isolated from the cells using 1 ml Trizol Reagent (Invitrogen) per 10 cm² cultivation area and followed by further purification with the RNeasy Mini Kit (Qiagen). RNA isolation and purification were performed according to the manufacturer's instructions. RNA concentrations and the ratio of A260:A280 were determined with a NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Thermo Fisher Scientific). The isolated RNA was subjected to complementary DNA synthesis. Reverse transcription was done according to the manufacturer's instructions using SuperScript III and random primers (Invitrogen). The concomitant relative gene transcript analyses were done with quantitative real-time PCR and using specific TaqMan[®] Gene Expression Assays (Applied Biosystems). The adipokines analysed for gene transcript amount were PPARγ (Hs01115512_m1), IL-6 (Hs00174131_m1), leptin (Hs00174877_m1), TNF-α (Hs00174128_m1), hypoxia-inducible factor-1, α subunit (HIF-1α, Hs00153153) and adiponectin (Hs00605917_m1). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH, Hs99999905_m1) was used as a control. All assays were run in triplicate from each sample using a 7500 FAST Real-Time PCR System (Applied Biosystems).

Measurement of adipokines by ELISA

The total amount of leptin and IL-6 protein in cell-culture media was measured with commercial Human Quantikine ELISA kits (R&D Systems). The assays were conducted according to the manufacturer's instructions and ninety-six-well microplates were analysed with a SpectraMAX 250 Microplate Spectrophotometer (Molecular Devices Company).

Data analysis and statistics

Quantitative PCR results were analysed with the delta-delta method, i.e. the relative quantification method⁽²²⁾ describing the change in the expression of the target gene transcript in a treatment group relative to a reference group. The amount of the target gene transcript was also normalised to an internal control gene, *GAPDH*^(23,24). The calibrator, i.e. the control group, was a medium-only treatment both with and without hypoxic conditions. No significant changes in *GAPDH* gene transcript amount were found between the different treatment groups. The gene transcription results are reported as the mean relative mRNA level (also referred to as fold difference, FD) and the ELISA results as protein release (pg/ml). The statistical significances of the differences between the different treatments were determined by ELISA analysis using one-way ANOVA and Tukey's multiple comparison test. For gene transcription analysis, Student's *t* test with a two-tailed distribution



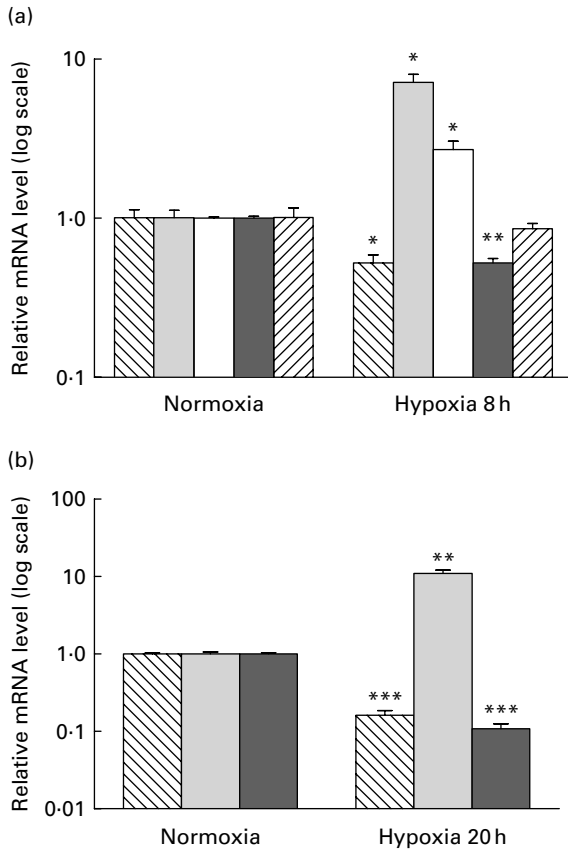


Fig. 1. Relative adipokine mRNA expression levels in human adipocytes after (a) a short-term (8h) and (b) a long-term (20h) hypoxia induction. The relative mRNA levels of PPAR γ (▨), leptin (□), IL-6 (■), TNF- α (■) and adiponectin (▤) were measured by quantitative PCR after the indicated induction times. Values are means, with their standard errors represented by vertical bars (n 3). Mean values were significantly different from those of normoxia: * P <0.05, ** P <0.01, *** P <0.001 (Student's t test).

was assessed. P values of 0.05 or less were considered to be statistically significant.

Results

Effects of hypoxia on adipokine mRNA expression

To measure the effect of low (1%) oxygen conditions on human omental adipocytes and to test the competence and reliability of the hypoxia stress model, changes in the gene transcription of adipokines were measured with real-time quantitative PCR. The relative mRNA levels of leptin and IL-6 increased in adipocytes in hypoxia while the mRNA level of PPAR γ decreased (Fig. 1(a)). Hypoxia also decreased the relative mRNA level of TNF- α . After 8 h hypoxia, the expression of leptin mRNA was increased 7-fold (FD=7.1, P =0.014) and the expression of IL-6 mRNA 3-fold (FD = 2.7, P =0.032). The relative mRNA level of both PPAR γ (P =0.030) and TNF- α (P =0.009) showed a FD of 0.52 after 8 h hypoxia, i.e. a 2-fold decrease. The 8 h hypoxia induction did not have any significant effect on the mRNA level of adiponectin. After 20 h in hypoxia, the expression levels changed even more dramatically; the leptin mRNA level increased 11-fold (FD = 11.0,

P =0.006), and PPAR γ and TNF- α mRNA levels decreased 6-fold (FD = 0.16, P =0.0002) and 9-fold (FD = 0.11, P =0.0001), respectively (Fig. 1(b)). Adiponectin mRNA expression was not measured after 20 h hypoxia. The results of the IL-6 mRNA expression level varied after long hypoxia incubation. The extended hypoxia treatments (20h) also caused a decrease in the RNA yield from the adipocytes as a consequence of the stress caused by hypoxia, thus enough RNA could not be collected for over 20 h hypoxia measurements. Cell viability or apoptosis was not measured, but the morphology of the cells was clearly altered after the 24 h hypoxia treatment (microscopic evaluation, data not shown). HIF-1 α mRNA expression was measured from the differentiated adipocytes before and after the hypoxic stress, and it decreased significantly after 4 h hypoxia (P =0.0008, data not shown).

Effects of betaine on adipocyte inflammation

The effect of betaine on the mRNA expression of the inflammatory markers IL-6 and leptin was tested in adipocytes in normoxic (21% O $_2$) and hypoxic conditions (1% O $_2$). In normoxic conditions, betaine (250 μ mol/l) decreased the mRNA

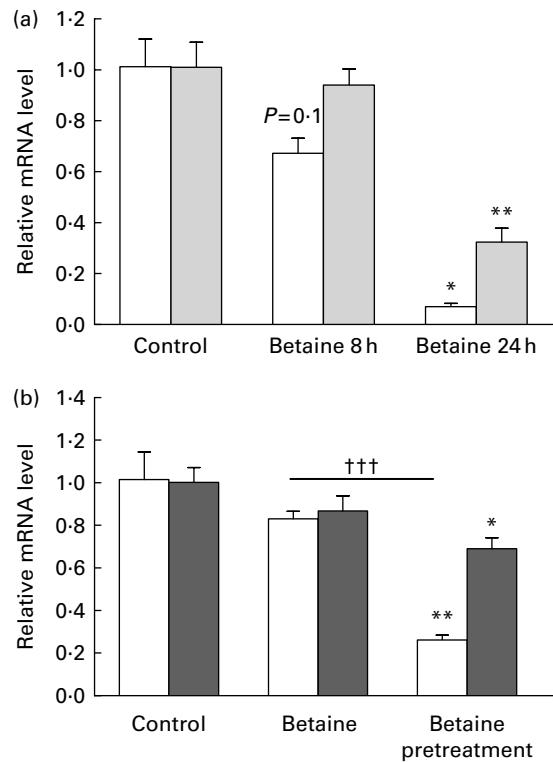


Fig. 2. Effect of betaine (250 μ mol/l) on adipokine mRNA expression in human adipocytes in (a) normoxia and (b) hypoxia. The relative mRNA levels of IL-6 (□), leptin (■) and TNF- α (■) were measured by quantitative PCR after 8 or 24 h in normoxia and after 8 h in hypoxia. Betaine was administered to the cell culture either simultaneously with or 16 h before hypoxia induction, the latter indicated as a pretreatment. Values are means, with their standard errors represented by vertical bars (n 3). Mean values were significantly different from those of the appropriate medium control: * P <0.05, ** P <0.01 (student's t test). ††† Mean values were significantly different between the indicated treatments (P <0.001; Student's t test).

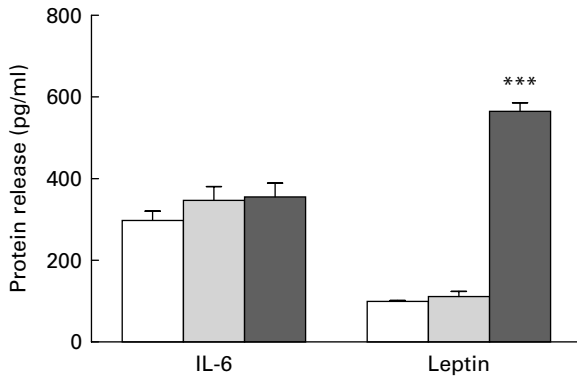


Fig. 3. Effect of hypoxia (8–16 h) on adipokine secretion by human adipocytes. The protein release of IL-6 and leptin is expressed as pg/ml. Values are means, with their standard errors represented by vertical bars (n 3). *** Mean values were significantly different from that of the appropriate medium control (□) ($P < 0.001$; one-way ANOVA). □, Hypoxia 8 h; ■, hypoxia 16 h.

expression of both adipokines (Fig. 2(a)). After 24 h induction with betaine, the mRNA expression level of IL-6 decreased 14-fold ($FD = 0.07$, $P < 0.05$) and that of leptin 3-fold ($FD = 0.32$, $P < 0.01$). In normoxia, betaine did not have any significant effect on the mRNA expression level of PPAR γ or TNF- α (data not shown).

In hypoxic conditions, betaine decreased the mRNA expression of the pro-inflammatory adipokines IL-6 and TNF- α compared with the hypoxia-induced samples without any additional betaine treatment (Fig. 2(b)). This inflammation-reducing effect was significant when betaine was administered to the cell culture as a pretreatment 16 h before the succeeding 8 h hypoxia induction; the relative mRNA levels of IL-6 and TNF- α showed a 4-fold ($FD = 0.26$, $P < 0.01$) and 1.5-fold ($FD = 0.69$, $P < 0.05$) decrease, respectively. Betaine pretreatment significantly fortified the inflammation-reducing effect of betaine ($P < 0.001$; Fig. 2(b)).

Effects of hypoxia and betaine on adipokine secretion

The secretion of selected adipokines into the cell-culture medium was measured with specific ELISA. Under hypoxic conditions (1% O $_2$), an induced secretion of IL-6 and leptin was observed. There was a 1.5-fold significant increase ($P < 0.01$) in the release of IL-6 after 20 h exposure to hypoxia, but not earlier (Figs. 3 and 4(a)). On the other hand, a significant 5.7-fold increase ($P < 0.001$) in the release of leptin was detected after 16 h hypoxia induction, and the level of secreted leptin was even higher after a 20 h induction (a 17-fold increase compared with the control, $P < 0.001$) (Figs. 3 and 4(b)). A beneficial effect of betaine on inflammation-related adipokine protein release was detected in hypoxia after 20 h (Fig. 4). A slight non-significant decrease in secreted IL-6 was observed with betaine concentrations of 50 and 250 $\mu\text{mol/l}$, when compared with hypoxia treatment without betaine (Fig. 4(a)). The highest dose of betaine (500 $\mu\text{mol/l}$) appeared not to have any additional benefits to the release of IL-6. Betaine also decreased the secretion of leptin after the 20 h induction in hypoxia (Fig. 4(b)), and

a statistically significant decrease (1.3-fold, $P < 0.001$) was measured with the highest betaine dose (500 $\mu\text{mol/l}$).

Discussion

Earlier cross-sectional surveys have indicated an improved effect of orally ingested betaine on low-grade inflammation. In a study by Detopoulou *et al.*⁽²⁵⁾, a high dietary intake of betaine resulted in lower concentrations of the inflammation markers TNF- α and C-reactive protein in human blood. Our experiments show that betaine lowered the inflammatory status in adipocytes suffering from hypoxic conditions. The present results show that hypoxia increased the expression and secretion of the pro-inflammatory marker IL-6, and that physiologically relevant concentrations of betaine reduced this effect. Under hypoxia, betaine also reduced the expression of the pro-inflammatory marker TNF- α . Hence, betaine reduced the effects of inflammation caused by hypoxia in human adipocytes. Betaine induced a clear decrease in IL-6 mRNA expression also in normoxia, implying that betaine also has a potential inflammation-reducing effect during normal adiposity.

The hypoxia stress model with human adipocytes was shown to respond as expected, and the results of gene

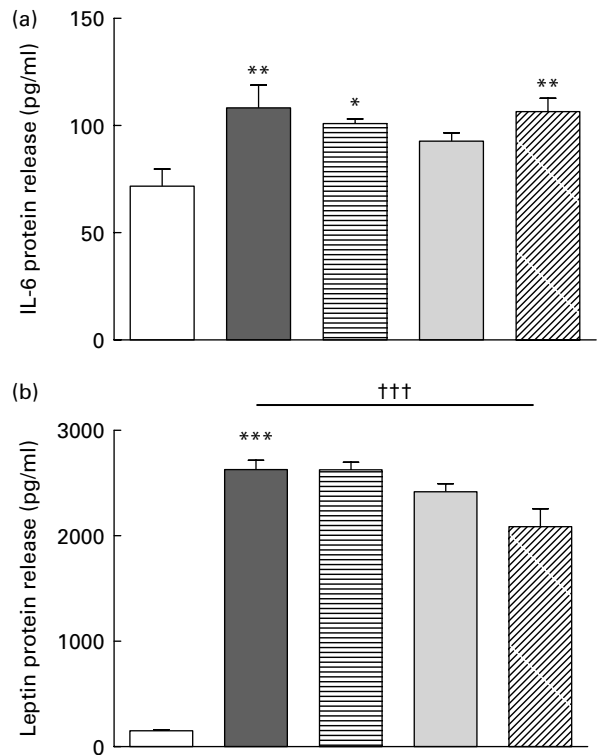


Fig. 4. Effect of betaine (50, 250 and 500 $\mu\text{mol/l}$) on adipokine secretion by human adipocytes in hypoxia (20 h). The protein release of (a) IL-6 and (b) leptin was measured with ELISA and expressed as pg/ml. Values are means, with their standard errors represented by vertical bars (n 3). Mean values were significantly different from those of the control (□): * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, ††† Mean values were significantly different between the indicated treatments ($P < 0.001$; one-way ANOVA). ■, Hypoxia; ▨, hypoxia + betaine 50 $\mu\text{mol/l}$; ▩, hypoxia + betaine 250 $\mu\text{mol/l}$; ▩, hypoxia + betaine 500 $\mu\text{mol/l}$.

transcription analysis are in line with previously reported literature. The expression of a central inducer of adipocyte differentiation, PPAR γ , is known to decrease in hypoxia⁽²⁶⁾, and the present data are in agreement with this, showing the diminished amount of PPAR γ mRNA in the differentiated adipocytes after hypoxia. Hypoxia is known to inhibit the differentiation process of adipocytes⁽²⁷⁾ and the diminished PPAR γ expression in a hypoxic environment serves as one mechanistic explanation for this⁽²⁸⁾. PPAR γ is activated by an agonist ligand in fibroblastic cells stimulating a full cascade of differentiation, including morphological changes, lipid accumulation and the expression of several genes characteristic of fat cells⁽¹⁰⁾. Hence, the overall expression of PPAR γ in normoxia suggests the successful differentiation of human preadipocytes used in the present experiment.

The expression and secretion of leptin, both of which occur principally in mature adipocytes⁽²⁹⁾, increase as a consequence of hypoxic conditions⁽⁷⁾. We examined here the expression of leptin mRNA, which was detected in both normoxia and hypoxia. Leptin is known to be essentially undetectable in preadipocytes in normoxia⁽²⁹⁾, hence the presence of leptin mRNA in normoxia demonstrates the successful differentiation of human preadipocytes. The leptin mRNA level showed, as expected, a clear increase under hypoxic stress. In addition to the effect of low oxygen tension on transcription processes in adipocytes, a part of the vast increase in leptin mRNA after hypoxic treatment could be explained by the fact that the exposure of preadipocytes to 1% O₂ can also result in the induction of leptin expression⁽²⁶⁾. Most of the preadipocytes matured into adipocytes after the 14 d differentiation period, but in the cell culture, some preadipocytes may have remained whose leptin production would have started only after hypoxic stress.

In hypoxia, the gene expression and secretion of IL-6, a pro-inflammatory protein, increase in adipocytes⁽⁷⁾. This observation was also measurable in our experiments with the adipocyte model, where hypoxia induction increased the mRNA levels of IL-6. The observed increased IL-6 transcript amount in short-term hypoxia was not reflected in the protein amount, but the increase in IL-6 protein amount was observed in extended (over 20 h) hypoxia, during which the IL-6 transcript amount was not clearly affected. It is known that the mRNA transcript levels can only give an estimation of the gene product and its function, and therefore further analyses of functional protein abundance are needed to fully investigate the effects on the whole process of gene expression. Adipose tissue IL-6 expression and circulating levels of IL-6 protein are also known to positively correlate with obesity, impaired glucose tolerance and insulin resistance^(30,31), suggesting that a preventive or reducing effect on IL-6 expression could potentially help to reduce the risk of such obesity-related diseases.

Another pro-inflammatory marker, TNF- α , in addition to being highly autocatalytic, is known to also stimulate the expression of several other cytokines⁽³²⁾. In our experiments, TNF- α resulted in a decreased amount of the gene transcript after hypoxic stress. In the literature, the effect of hypoxia on TNF- α levels has been reported to be inconsistent in

in vitro experiments. While some studies have shown an increase in TNF- α expression after hypoxic treatment⁽³⁾, others have not reported any significant effects at all⁽⁷⁾. In the present study, variable responses were also obtained, mostly showing no effects while in some experiments, the expression of TNF- α was also reduced. The variable results from past and present studies suggest that TNF- α may not necessarily be up-regulated under hypoxia in human adipocytes.

Several studies have shown that circulating levels of leptin are closely correlated with body fat mass and fat cell size^(33,34). Notwithstanding, most obese individuals do not respond to the increased leptin levels with an adequate reduction in food intake⁽³⁵⁾. Since there were indications that betaine could lower leptin gene transcription and secretion in adipocytes in both normoxic and hypoxic conditions, it could be hypothesised that betaine may have a role in normalising the high leptin levels in obese subjects. Further experiments with an obese animal model are required to study this hypothesis. Leptin gene transcription in hypoxia is known to be regulated transcriptionally by HIF-1⁽³⁶⁾, and therefore, it would be interesting to see whether the effects disclosed by betaine are also mediated through it. However, in the present study, the role of HIF-1 α was not studied in depth and further investigations would be needed in order to fully characterise the effect of betaine in hypoxia.

Betaine is known to work as an osmolyte in various cell types. In rat liver macrophages, betaine has shown to modulate immune function by restoring cell volume homeostasis during phagocytosis⁽³⁷⁾. As a methyl donor, betaine can also reduce homocysteine levels in adipose tissues, which can contribute to the improved adiponectin production and thus reduced inflammation in experimental animal models⁽³⁸⁾.

The beneficial effects of betaine supplementation on non-alcoholic fatty liver disease have been reported recently⁽¹⁹⁾. Long-term exposure to a high-fat diet can induce fatty liver and other liver injuries associated with obesity, insulin resistance and adipokine dysfunction⁽¹⁹⁾. Dysregulated adipokine production, especially elevated leptin expression, together with insulin resistance has been shown to be associated with the pathogenesis of non-alcoholic fatty liver disease and therefore the potentially normalising effect of betaine supplementation on leptin levels can be an important physiological factor in maintaining liver health.

The aim of the present study was to test the effect of betaine on the inflammatory response of adipocytes *in vitro* and to assess the usefulness of the hypoxia stress model of human adipocytes in research targeting the health effects of dietary supplements. One of the limitations of the present study is the fact that preadipocytes were obtained from a single donor. We chose this approach in order to minimise the number of variables in the study and to focus on the effects of betaine alone. We show that betaine may reduce inflammation in adipose tissue in hypoxia and may therefore potentially help to reduce systemic inflammation and the risk for other obesity-related diseases. Further investigations to assess the efficacy of betaine in reducing obesity-related inflammation *in vivo* are warranted.

Acknowledgements

This study was funded by DuPont. Lauri Naski and Henri Ahokoski are thanked for their skilful support in cell-culture and ELISA analyses, respectively. Heli Putaala, John Cowasji and Sofia Forssten are thanked for their help in image processing, proofreading the manuscript and reference managing, respectively. K. O. designed and performed the cell culture work and data analysis, and was responsible for the writing of the manuscript. K. O., S. L., N. R. and K. T. contributed to the study design, interpretation of the results and writing of the manuscript. The authors are employed by a manufacturer of betaine, but declare no other conflict of interest regarding this study.

References

- Ahima RS (2006) Adipose tissue as an endocrine organ. *Obesity* **14**, 242S–249S.
- Hosogai N, Fukuhara A, Oshima K, *et al.* (2007) Adipose tissue hypoxia in obesity and its impact on adipocytokine dysregulation. *Diabetes* **56**, 901–911.
- Ye J, Gao Z, Yin J, *et al.* (2007) Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. *Am J Physiol Endocrinol Metab* **293**, E1118–E1128.
- Pasarica M, Sereda MR, Redman LM, *et al.* (2009) Reduced adipose tissue oxygenation in human obesity: evidence for rarefaction, macrophage chemotaxis, and inflammation without an angiogenic response. *Diabetes* **58**, 718–725.
- Trayhurn P, Wang B & Wood IS (2008) Hypoxia and the endocrine and signalling role of white adipose tissue. *Arch Physiol Biochem* **114**, 267–276.
- Trayhurn P & Wood IS (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr* **92**, 347–355.
- Wang B, Wood IS & Trayhurn P (2007) Dysregulation of the expression and secretion of inflammation-related adipokines by hypoxia in human adipocytes. *Pflugers Arch* **455**, 479–492.
- Hauner H (2005) Secretory factors from human adipose tissue and their functional role. *Proc Nutr Soc* **64**, 163–169.
- Trayhurn P, Wang B & Wood IS (2008) Hypoxia in adipose tissue: a basis for the dysregulation of tissue function in obesity? *Br J Nutr* **100**, 227–235.
- Rosen ED & Spiegelman BM (2006) Adipocytes as regulators of energy balance and glucose homeostasis. *Nature* **444**, 847–853.
- Rajala MW & Scherer PE (2003) Minireview: the adipocyte—at the crossroads of energy homeostasis, inflammation, and atherosclerosis. *Endocrinology* **144**, 3765–3773.
- Zeisel SH, Mar M-H, Howe JC, *et al.* (2003) Concentrations of choline-containing compounds and betaine in common foods. *J Nutr* **133**, 1302–1307.
- Slow S, Donaggio M, Cressey PJ, *et al.* (2005) The betaine content of New Zealand foods and estimated intake in the New Zealand diet. *J Food Compos Anal* **18**, 473–485.
- Kamal-Eldin A, Larke HN, Knudsen KEB, *et al.* (2009) Physical, microscopic and chemical characterisation of industrial rye and wheat brans from the Nordic countries. *Food Nutr Res* **53**, article 14.
- Craig SA (2004) Betaine in human nutrition. *Am J Clin Nutr* **80**, 539–549.
- Lever M & Slow S (2010) The clinical significance of betaine, an osmolyte with a key role in methyl group metabolism. *Clin Biochem* **43**, 732–744.
- Eklund M, Bauer E, Wamatu J, *et al.* (2005) Potential nutritional and physiological functions of betaine in livestock. *Nutr Res Rev* **18**, 31–48.
- Fernandez-Figares I, Wray-Cahen D, Steele NC, *et al.* (2002) Effect of dietary betaine on nutrient utilization and partitioning in the young growing feed-restricted pig. *J Anim Sci* **80**, 421–428.
- Wang Z, Yao T, Pini M, *et al.* (2010) Betaine improved adipose tissue function in mice fed a high-fat diet: a mechanism for hepatoprotective effect of betaine in nonalcoholic fatty liver disease. *Am J Physiol Gastrointest Liver Physiol* **298**, G634–G642.
- Song Z, Deaciuc I, Zhou Z, *et al.* (2007) Involvement of AMP-activated protein kinase in beneficial effects of betaine on high-sucrose diet-induced hepatic steatosis. *Am J Physiol Gastrointest Liver Physiol* **293**, G894–G902.
- Schwab U, Törrönen A, Meririnne E, *et al.* (2006) Orally administered betaine has an acute and dose-dependent effect on serum betaine and plasma homocysteine concentrations in healthy humans. *J Nutr* **136**, 34–38.
- Livak KJ & Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. *Methods* **25**, 402–408.
- Gorzelnik K, Janke J, Engeli S, *et al.* (2001) Validation of endogenous controls for gene expression studies in human adipocytes and preadipocytes. *Horm Metab Res* **33**, 625–627.
- Ouchi N, Kihara S, Funahashi T, *et al.* (2003) Reciprocal association of C-reactive protein with adiponectin in blood stream and adipose tissue. *Circulation* **107**, 671–674.
- Detopoulou P, Panagiotakos DB, Antonopoulou S, *et al.* (2008) Dietary choline and betaine intakes in relation to concentrations of inflammatory markers in healthy adults: the ATTICA study. *Am J Clin Nutr* **87**, 424–430.
- Wang B, Wood IS & Trayhurn P (2008) Hypoxia induces leptin gene expression and secretion in human preadipocytes: differential effects of hypoxia on adipokine expression by preadipocytes. *J Endocrinol* **198**, 127–134.
- Zhou S, Lechpammer S, Greenberger JS, *et al.* (2005) Hypoxia inhibition of adipocytogenesis in human bone marrow stromal cells requires transforming growth factor- β /Smad3 signaling. *J Biol Chem* **280**, 22688–22696.
- Yun Z, Maecker HL, Johnson RS, *et al.* (2002) Inhibition of PPAR gamma 2 gene expression by the HIF-1-regulated gene DEC1/Stra13: a mechanism for regulation of adipogenesis by hypoxia. *Dev Cell* **2**, 331–341.
- Wang B, Jenkins JR & Trayhurn P (2005) Expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture: integrated response to TNF-alpha. *Am J Physiol Endocrinol Metab* **288**, E731–E740.
- Fernandez-Real JM & Ricart W (2003) Insulin resistance and chronic cardiovascular inflammatory syndrome. *Endocr Rev* **24**, 278–301.
- Tiihonen K, Ouwehand AC & Rautonen N (2010) Effect of overweight on gastrointestinal microbiology and immunology: correlation with blood biomarkers. *Br J Nutr* **103**, 1070–1078.
- Wang B & Trayhurn P (2006) Acute and prolonged effects of TNF-alpha on the expression and secretion of inflammation-related adipokines by human adipocytes differentiated in culture. *Pflugers Arch* **452**, 418–427.



33. Considine RV, Sinha MK, Heiman ML, *et al.* (1996) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med* **334**, 292–295.
34. Friedman JM & Halaas JL (1998) Leptin and the regulation of body weight in mammals. *Nature* **395**, 763–770.
35. Frederich RC, Hamann A, Anderson S, *et al.* (1995) Leptin levels reflect body lipid-content in mice – evidence for diet-induced resistance to leptin action. *Nat Med* **1**, 1311–1314.
36. Ambrosini G, Nath AK, Sierra-Honigmann MR, *et al.* (2002) Transcriptional activation of the human leptin gene in response to hypoxia. Involvement of hypoxia-inducible factor 1. *J Biol Chem* **277**, 34601–34609.
37. Warskulat U, Zhang F & Haussinger D (1996) Modulation of phagocytosis by anisoosmolarity and betaine in rat liver macrophages (Kupffer cells) and RAW 264.7 mouse macrophages. *FEBS Lett* **391**, 287–292.
38. Song ZY, Zhou ZX, Deaciuc I, *et al.* (2008) Inhibition of adiponectin production by homocysteine: a potential mechanism for alcoholic liver disease. *Hepatology* **47**, 867–879.