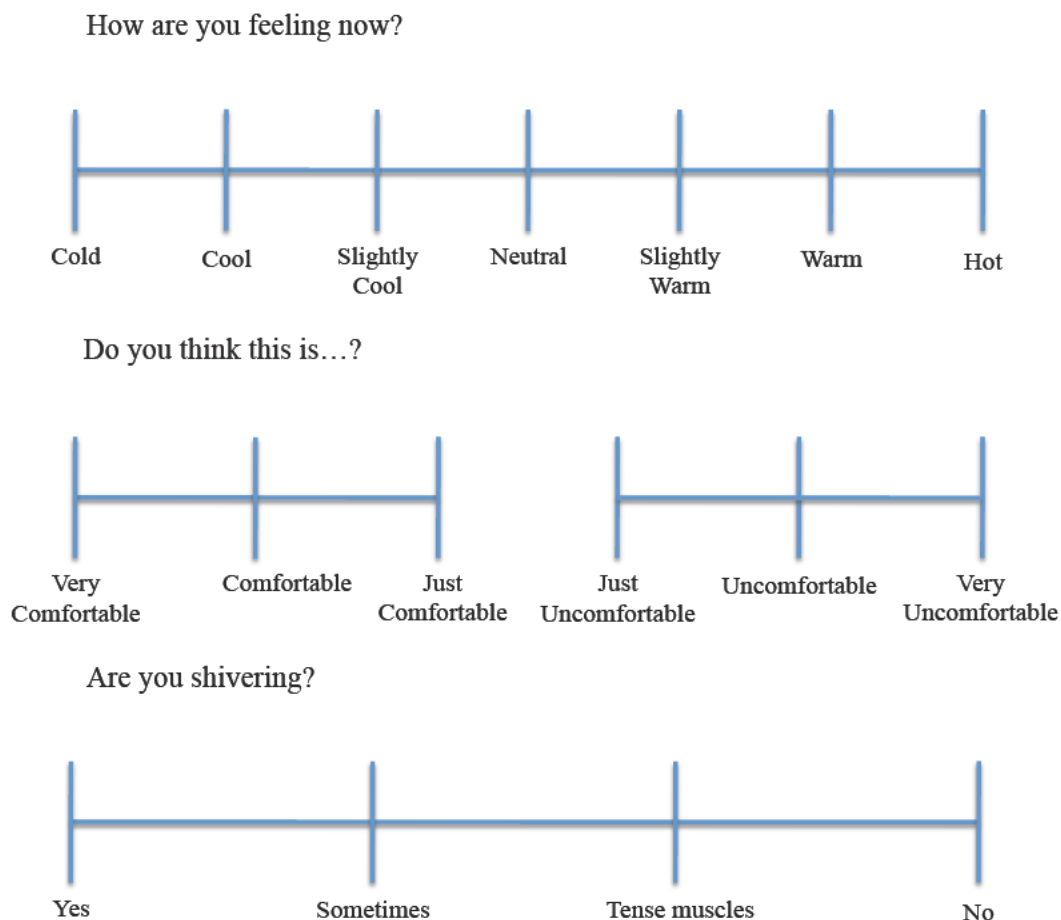## Supplemental methods

On prescribed time points (t=0, t=20, t=40, t=60, t=90, t=120, t=180, t=240, t=300 and t=360) after entering the cold room, subjects completed visual analogue scales (VAS-scales), on sensation, thermal comfort and shivering (Supplemental Figure 1). On the scale they were asked to indicate their sensation (How are you feeling now?), their comfort (Do you think this is…?) and shivering was reported (Are you shivering?). The scores were transferred to numeric data (from 0-10 for sensation, from -3.1 to -0.1 and 0.1 to 3.1 for comfort and 0-10 for shivering), so that analyses could be done. Incremental area under the curve was calculated for day 3 (6 hours) and day 10 (6 hours) and these values were compared by means of a paired sample t test.

How are you feeling now?

| Cold | Cool | Slightly Cool | Neutral | Slightly Warm | Warm | Hot |

Do you think this is…?

| Very Comfortable | Comfortable | Just Comfortable | Just Uncomfortable | Uncomfortable | Very Uncomfortable |

Are you shivering?

| Yes | Sometimes | Tense muscles | No |

*Supplemental Figure 1: **Visual representation of the VAS-scales on sensation, overall comfort and shivering.** The questionnaires were presented in either Dutch or English to the subjects through an Internet browser.*

mRNA expression levels of several markers were measured both in subcutaneous white fat samples and in skeletal muscle material. No effect of the cold acclimation was found in fat tissue, however PPARgamma and adiponectin were up regulated in the skeletal muscle. The following Real-Time RT-PCR Primer Sequences were used;

Supplemental Table 1: Real-Time-RT-PCR Primer Sequences used for gene expression in both skeletal muscle and white adipose tissue.

Genes	Sequences
hUCP1	AGGTCCAAGGTGAATGCCC GCGGTGATTGTTCCCAGGA
hCIDEA	TCCGGGTCTCCAACCATGA GGCATCCAGAGTCTTGCTGAT
hPRDM16	AGACTTCGGATGGGAGCAAAT TCCACGCAGAACTTCTCACTG
hPGC1a	TCTGAGTCTGTATGGAGTGACAT CCAAGTCGTTCACATCTAGTTCA
hAP2	ACTGGGCCAGGAATTTGACG CCCCATCTAAGGTTATGGTGCTC
hPPARgamma	ACCAAAGTGCAATCAAAGTGGA AGGCTTATTGTAGAGCTGAGTCT
hAdiponectin	TATCCCCAACATGCCCATTCG TAGGCAAAGTAGTACAGCCCA
hAdipsin	GACACCATCGACCACGACC GCCACGTCGCAGAGAGTTC
hLeptin	TGCCTTCCAGAAACGTGATCC CTCTGTGGAGTAGCCTGAAGC
hFNDC5	AAGCACAAGGACTGACTCAAGC CATGTCCTTGATGGCTGGAT
hTMEM26	ATGGAGGGACTGGTCTTCCTT CTTCACCTCGGTCACTCGC
hCD137	AGCTGTTACAACATAGTAGCCAC TCCTGCAATGATCTTGTCCTCT
hTBP	CCACTCACAGACTCTCACAAC CTGCGGTACAATCCCAGAACT
hPPIA	TGGTGTTTGGCAAAGTGAAA TCGAGTTGTCCACAGTCAGC
hB2M	GTGCTCGCGCTACTCTCTCT TCTCTGCTGGATGACGTGAG

Supplemental Table 2: mRNA expression in skeletal muscle and white adipose tissue.

	SKELETAL MUSCLE		WAT	
	PRE	POST	PRE	POST
UCP1	1.14 ± 0.61	1.37 ± 0.83	0.79 ± 0.71	0.61 ± 0.56
CIDEA	1.12 ±0.67	1.10 ± 0.79	1.13 ± 0.50	1.23 ± 0.66
PRDM16	1.14 ± 0.43	1.45 ± 1.01	1.06 ± 0.29	0.97 ± 0.27
PGC1a	1.03 ± 0.25	1.14 ± 0.37	1.11 ± 0.49	0.98 ± 0.33
AP2	1.03 ± 0.25	1.03 ± 0.39	1.06 ± 0.34	1.04 ± 0.32
PPARgamma	1.03 ± 0.25	1.19 ± 0.34*	1.06 ± 0.32	1.11 ± 0.23
ADIPONECTIN	1.07 ± 0.71	1.76 ± 1.42*	1.07 ± 0.38	0.98 ± 0.17
ADIPSIN	1.08 ± 0.40	1.00 ± 0.43	1.09 ± 0.37	1.10 ± 0.31
LEPTIN	1.08 ± 0.44	1.38 ± 0.91	1.16 ± 0.51	1.07 ± 0.73
FNDC5	1.04 ± 0.30	0.97 ± 0.35	1.04 ± 0.19	1.06 ± 0.34
TMEM26	1.10 ± 0.38	1.02 ± 0.40	1.12 ± 0.44	1.31 ± 0.46
CD137	1.20 ± 0.76	1.58 ± 1.08	1.12 ± 0.83	0.80 ± 0.45

Results are expressed as means ± SD. * $p < 0.05$ PRE vs. POST. All expression levels are normalized to the average of all subjects PRE cold. The effect of the cold acclimation was tested with a paired samples t test.