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Heat Stress Response and Inflammation in Acute Temperature Stresses

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HEAT STRESS RESPONSE AND INFLAMMATION IN ACUTE TEMPERATURE STRESSES

by

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Resume

Inflammatory factors, both centrally produced and released from adipocytes (fat cells), have been shown to affect metabolism and to contribute to the pathogenesis and development of many chronic diseases such as heart disease, type 2 diabetes and cancer. Inflammatory response has been shown to be affected by environmental stresses such as heat and cold exposure. The aim of this thesis was to study the effects of heat and cold stresses on inflammatory response over the course of 3 experiments which exposed healthy subjects to one of two heat intensities or to cold stress. This thesis is presented in the classical format and is composed of the following: Chapter 1 introduces the main concepts of inflammation and summarizes the existing literature on temperature stress and inflammation; Chapter 2 describes the methodologies used; Chapter 3 presents the results from the three studies; Chapter 4 discusses the relevance of our results in terms of the pre-existing research in this area and Chapter 5 provides some speculation into the underlying causes of the observed changes, suggests further studies to investigate the speculations and ends with some concluding remarks.

Subjects were exposed to either moderate intensity heating (Experiment 1: three hours in chamber set at 42°C, 1.1±0.01°C core temperature increase), low intensity heating (Experiment 2: two hours in liquid perfusion suit with circulating water temperature set at 48°C, 0.4±0.02°C core temperature increase) or cold exposure (Experiment 3: two hours in liquid perfusion suit with circulating water temperature set at 5°C, no change in core temperature). Fasting blood samples were taken before and during the temperature stress. Plasma levels of pro-inflammatory factors, interleukin-6

(IL-6) and tumor necrosis factor α (TNFα) were measured along with the adipocyte-derived anti-inflammatory factor, adiponectin.

Results showed that, during the higher intensity heat exposure, plasma IL-6 increased by 440% while no statistical change were observed for adiponectin and TNFa levels. No changes were noted in inflammatory markers during the lesser intensity heating. With cold exposure, plasma IL-6 and adiponectin increased over time, falling short of statistical significance. No change in TNFα was noted at any point. Increases in plasma IL-6 and adiponectin are positively correlated with increases in core temperature.

This thesis supports the notion that temperature stress influences inflammatory response. The novelty of this work lies in the use of two different heat exposure intensities, both lower than the majority of heating intensities used in previous literature, the study of cold stress in human subjects without the confounding effect of other co-existing stresses and the quantification of adiponectin levels in human subjects during temperature stress.

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Chapter 1 – Introduction

Inflammation

Inflammation is defined as the body's response to injury, illness, toxins or environmental stressors (Vander et al. 1998). The inflammatory response activates the immune system in response to internal and external stresses and involves the integration of many complex signals in distinct cells and organs (Gabay and Kushner. 1999). Inflammation can either be acute and self-limited, as seen during a transient illness or injury, or chronic, due to an ongoing trigger (Rote. 2002b). The long term consequences of prolonged inflammation are often associated with several chronic disease states (Woods et al. 2006).

Inflammatory factors are the chemical mediators of inflammation (Rote. 2002a; Vander et al. 1998). Most inflammatory factors are cytokines, small molecules that have multiple signaling roles and actions which appear to be redundant between cytokines (Dinarello. 2000). Traditionally inflammatory factors have been categorized as having either pro-inflammatory or anti-inflammatory characteristics. However, many of these factors are now seen to be complex in nature, with their effects being multipotent depending on local biochemical environments, enabling the same factor to have both pro-inflammatory and anti-inflammatory tendencies (Pedersen. 2007). The terms pro-inflammatory and anti-inflammatory will be used in this document because they are still in common use in the current literature but the reader should be aware that as more is uncovered regarding the factors that are part of the inflammatory response, their roles become less straightforward. Pro-inflammatory cytokines, such as interleukins (IL) 6, 1β, 2, 8, 12 and 15, tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ) and transforming growth factor β (TGF- β) have been typically shown to stimulate

inflammation. Anti-inflammatory factors, including interleukin-10 (IL-10) and adiponectin, typically inhibit the inflammatory response (Rote. 2002a). Table 1.1 summarizes the pro and anti-inflammatory factors mentioned in this document.

The acute phase response is a rapid system-wide response to any injury ranging from a tiny paper cut to a major cardiac infarct. This response is stimulated by the release of inflammatory factors: TNFα and IL-6 are two of major factors involved in stimulating this pathway (Gabay and Kushner. 1999). Despite its name, the acute phase response occurs with both acute and chronic inflammation. The acute phase response consists of changes in plasma protein concentrations as well as numerous associated behavioral, physiologic, biochemical and nutritional changes (Gabay and Kushner. 1999).

Sources of inflammation

The major systemic source of inflammation is from white blood cells, specifically, from macrophages and monocytes, which, as the main mediators for communication between the immune system and the rest of the body, can rapidly induce a system-wide inflammatory response (Angeli and Randolph. 2006). However, other tissues have also been shown to secrete inflammatory proteins. Though it is suspected that these inflammatory factors may be released as local signals, they can also have a systemic effect, particularly when large quantities are released (Juge-Aubry et al. 2005; Pond. 2005).

Table 1.1: A summary of pro and anti-inflammatory factors mentioned in this thesis.

Pro-inflammatory factors	Anti-inflammatory factors
IL-6	IL-10
TNFα	Adiponectin
IL-1β	
TGFβ	
IFNγ	
IL-8	
IL-2	
IL-12	
IL-15	

Adipose tissue

Adipose tissue has recently been shown to act as an endocrine organ and to release its own inflammatory factors from both adipocytes and from the macrophages that accumulate within adipose tissue (Scherer. 2006; Trayhurn and Wood. 2004; Trayhurn. 2005). Adipokines, the proteins and hormones secreted by adipocytes, can be classified as having either metabolic or inflammatory functions, with most factors having an overlap of both functions (Juge-Aubry et al. 2005). Adipokines include cytokines such as IL-6, IL-1 and TNFα that are released from multiple sources in the body (Juge-Aubry et al. 2005) as well as factors specific to adipose tissue, such as adiponectin and leptin (Scherer. 2006; Trayhurn and Wood. 2004; Trayhurn. 2005; Trujillo and Scherer. 2005).

The secretion of adipokines can be either directly (i.e. the release of many inflammatory cytokines such as IL-6 and TNFα) or inversely related to fat mass (i.e. the release of adiponectin) (Esposito et al. 2006; Trujillo and Scherer. 2005). These patterns have been demonstrated in epidemiological studies where body mass index (BMI), fat mass or weight gain in large cohorts has been negatively correlated with blood levels of adiponectin (Araneta and Barrett-Connor. 2007; Arita et al. 1999; Saltevo et al. 2007) and positively correlated with IL-6 and TNFα levels (Maachi et al. 2004; Park et al. 2005). Along the same line, follow-up studies of liposuction or dermolipectomy patients show decreased plasma IL-6 and TNFα and increased adiponectin levels following the removal of fat mass (Esposito et al. 2006; Giugliano et al. 2004; Rizzo et al. 2005).

Along with adipocytes, macrophages within adipose tissue are another source of adipokines, particularly in obesity (Wellen and Hotamisligil. 2003; Xu et al. 2003). Macrophage presence in adipose tissue has be shown to be greater in obese compared to

lean subjects and appears to decrease following weight loss (Cancello et al. 2005). The signal that attracts macrophages to adipose tissue in obesity is unclear but both increased levels of adipocyte-derived adipokines and the development of necrotic adipose tissue due to an insufficient blood supply are thought to be involved (Zeyda and Stulnig. 2007). These macrophages both contribute significantly to the rise in inflammatory factors seen in obesity and stimulate pro-inflammatory factor gene expression in adipocytes (Cancello et al. 2005; Zeyda and Stulnig. 2007). *In vitro* work has shown that adipocytes incubated in macrophage-conditioned media have an increased release of inflammatory factors as well as increased lipolysis and reduced insulin sensitivity (Permana et al. 2006). Adipocyte-derived pro-inflammatory cytokines could further stimulate the macrophages to release more pro-inflammatory factors, creating a vicious circle of chronic inflammation (Permana et al. 2006; Ruan et al. 2002) illustrated in Figure 1.1.

Skeletal muscle

Skeletal muscle has recently been shown to be a source of secreted inflammatory factors (Pedersen and Fischer. 2007a; Pedersen and Fischer. 2007b). These factors, known as myokines, to date include IL-6, IL-8 and IL-15 (Pedersen et al. 2007). The release of IL-6 and IL-8 appears to be triggered by muscle contractions whereas IL-15 release has been shown to increase with strength training (Nielsen and Pedersen. 2007). The roles of myokines are not well understood. IL-8 may be involved in promoting angiogenesis and IL-15 appears to have an anabolic effect and seems to reduce fat mass (Nielsen and Pedersen. 2007; Pedersen et al. 2007). Paradoxical to its above described

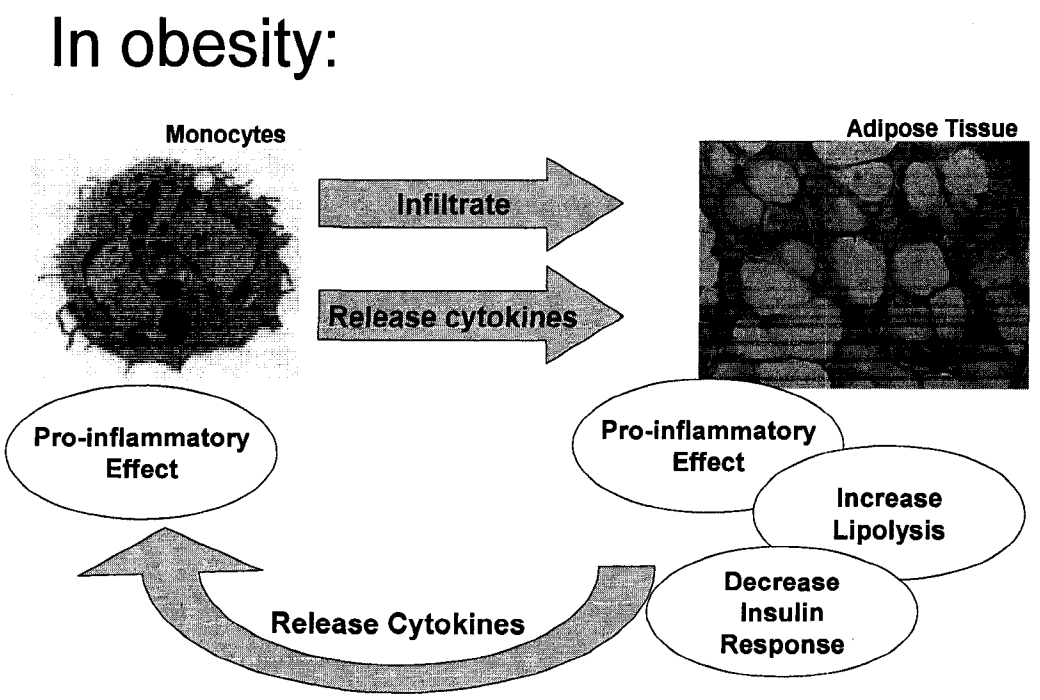

Figure 1.1: The vicious circle of inflammation in obesity. Monocytes infiltrating adipose tissue release cytokines which induce the release of pro-inflammatory cytokines from adipocytes. These cytokines further stimulate the monocyte-induced inflammatory response.

relationship with insulin resistance, at the level of skeletal muscle, IL-6 acutely released during muscle contraction appears to promote insulin sensitivity and glucose uptake (Al-Khalili et al. 2006; Pedersen and Fischer. 2007b; Ruderman et al. 2006; Steensberg et al. 2003; Weigert et al. 2005).

Other sources of inflammation

Any nucleated cell is potentially capable of secreting inflammatory factors: resulting in numerous potential sources of cytokines (Dinarello. 2000). Many sources are thought to act as local signals and to have a minimal contribution to systemic cytokine levels. Like with skeletal muscle, other sources, such as the brain (Nybo et al. 2002) and peritendon tissue (Langberg et al. 2002), have been shown to release inflammatory factors in response to exercise.

Stress and inflammation

Different forms of stress on the body have been shown to modify the inflammatory response (Tilders et al. 1999). Stresses can include such stimuli as psychosocial stress, hypoxia, exercise, illness and environmental conditions such as temperature. These stresses activate the sympathetic nervous system and cause neuroendocrine stimulation of hypothalamic-pituitary axis, leading to the stimulation and modification of inflammatory response (Walsh and Whitham. 2006).

Environmental stress

Among endotherms, changes in body temperature have been shown to affect inflammation and inflammatory response (Walsh and Whitham. 2006). This phenomenon has been explored and, though not fully understood on the physiological

level, has been used in therapeutic situations in attempt to modify inflammatory response, as seen in experimental uses of hyperthermia as an adjuvant cancer treatment (Manjili et al. 2002) and in the use of body cooling in trauma (Shiozaki et al. 1999), cardiac surgery (Birdi et al. 1999) and neurosurgical cases (Deng et al. 2003; Wang et al. 2006). What follows is a more detailed description of the effects that acute environmental temperature changes have on inflammation.

Heat exposure

Hyperthermia, or excessive heat gain, can be divided into 3 main categories (Walsh and Whitham. 2006): 1-) Fever is a cytokine-induced increase in core temperature, creating a new temperature equilibrium. The elevated body temperature in fever is widely accepted to directly affect immune and inflammatory response, assisting the body in defending itself against the infecting pathogens. 2-) Active hyperthermia develops during physical exercise when the body can not dissipate the excess heat produced during exercise, causing core temperature to increase. 3-) Passive hyperthermia occurs when an elevated environmental temperature causes core temperature to rise due to insufficient heat loss. Although all three hyperthermic conditions have been shown to affect inflammation and the immune system, here, the focus will be on passive hyperthermia in order to attempt to separate the inflammatory effect of heat exposure from the inflammatory effect of exercise or the pre-existent inflammatory state that is seen with fever.

Table 1.2 summarizes the existing data on the effects of heat exposure on cytokines in human studies (1.2A), animal studies (1.2B) and *in vitro* (1.2C) studies.

Table 1.2A: Effects of heat exposure on cytokines in humans

Study	Subjects	Heating Method	Duration	Core Temperature	Results
Downing and Taylor. 1987	N/A	Hot water immersion	Until core temperature of 39°C reached	39°C	Increased IL-2 release from isolated monocytes.
Park et al. 1990	2 advanced cancer patients who have not received chemotherapy in past year	Microwaves x3 sessions	Maximum temperature maintained 30-60 minutes.	Increased by 2°C	Baseline IL-1, IL-2 and IFNγ release from isolated peripheral blood mononucleocytes of cancer patients were lower than normal. Over the course of the 3 heat treatments, IL-1, IL-2 and IFNγ release increased.
Bouchama et al. 1991	17 heat stroke patients	Pilgrims to Mecca presenting to hospital with heat shock	N/A	42.1±0.2°C	Plasma TNFα, IL-1α, and lipopolysaccharide (LPS) were elevated on presentation in all heat stroke patients.
Kappel et al. 1991b	8 healthy men 22-35 years old	Hot water vs thermoneutral water immersion	2 hours	39.5°C	Blood mononuclear cells were isolated and treated with IL-2 or IFNα. IL-2 further induced natural killer cell activity in blood from hyperthermia subjects compared to thermoneutral conditions.
D'Oleire et al. 1993	9 cancer patients receiving whole body hyperthermia treatment.	N/A	Body temperature maintained for 60 minutes	41.8°C	Increased plasma IL-1β and TNFα following whole body hyperthermia.

Table 1.2A: Effects of heat exposure on cytokines in humans

Study	Subjects	Heating Method	Duration	Core Temperature	Results
Bouchama et al. 1993	28 (25 M, 3 F) heat stroke patients before and after cooling, 10 normal controls (7 M, 3 F; 37±9yo).	Pilgrims to Mecca presenting to hospital with heat shock	N/A	41.2±0.2°C	Elevated plasma CRP in 72% of heat shock patients. Elevated IL-6 in 100% of heat shock patients. Elevated IL-1β in 39% of heat shock patients. Elevated IFNγ in 50% of heat shock patients. IL-6 levels were positively correlated with severity of heat shock.
Robins et al. 1995	Cancer patients receiving chemotherapy and whole body hyperthermia. Control group received only chemotherapy.	Radiant heat device or extracorporal whole body hyperthermia.	Body temperature maintained for 60 minutes	41.8°C	Increased plasma IL-1β, IL-6, IL-8, IL-10 and TNFα over course of whole body hyperthermia.
Hammami et al. 1997	25 (19 M, 6 F; mean age 57±4) heat stroke patients before and after cooling, 14 heat stressed controls (mean age 51.8 +/- 8 yrs; male/female ratio of 2:3), 13 normal controls.	Pilgrams to Mecca presented to hospital with heat shock or heat stress.	N/A	42.3±0.2°C	Soluble tumor necrosis factor receptors 60 and 80 (sTNFR 60 and sTNFR 80) concentrations were greater in heat stressed control subjects than in normal controls. sTNFR 60 was further increased in heat shock. Soluble interleukin-6 receptor (sIL-6R) concentrations were elevated in heat stressed controls compared to heat shock and normal controls. TNFα was not detected at any time in any group. IL-6 was not detectable in normal controls and was significantly greater in heat shock subjects compared to heat stressed subjects. Significant positive Pearson's correlations were found between IL-6, sIL-6R, sTNFR 60 and sTNFR 80 and both temperature and creatinine.

Table 1.2A: Effects of heat exposure on cytokines in humans

Study	Subjects	Heating Method	Duration	Core Temperature	Results
Hashim et al. 1997	26 patients (8 M; 35-70 yo) heat stroke patients.	Pilgrims to Mecca presenting to hospital with heat stroke	N/A	Core body temperatures ranged from 40.8°C-43.28°C (median 42.38°C)	Serum IL-6 levels were elevated in all patients. Levels >900pg/ml on admission were associated with a decreased chance of survival. Soluble type 2 TNFα receptor levels were elevated in all patients but high levels were associated with mortality.
Bouchama et al. 2000	25 (19 M, 6 F; 57±4 yo) heat stroke patients, 13 normothermic heat-stressed subjects. 6 healthy subjects' monocytes from blood draw at 24°C	Pilgrams to Mecca presented to hospital with heat stroke / heat stress. Monocytes were incubated.	N/A for in vivo. 24h *in vitro* monocytes.	42.4±0.8°C rectal temperature in heat shock. Monocytes *in vitro*: 37, 39, 41 or 43 °C	Plasma IL-10 was elevated in hyperthermia patients and decreased with cooling. In monocytes, LPS-stimulated IL-10 release was successively lower with each increased temperature.
Atanackovic et al. 2002	11 cancer patients receiving whole body hyperthermia and chemotherapy; 5 patients receiving chemotherapy alone	Radiant heating device	60 minutes	41.8°C	Serum IL-6 and TNFα increased transiently with whole body hyperthermia and chemotherapy, but not with chemotherapy alone. 5 hyperthermia patients' CD8+ lymphocytes were assessed for intracellular cytokines: at 24 hours post-hyperthermia, IFNγ or TNFα was elevated in 4 out of 5 patients.

Table 1.2A: Effects of heat exposure on cytokines in humans

Study	Subjects	Heating Method	Duration	Core Temperature	Results
Ahlers et al. 2005	10 metastatic cancer patients (6 M, 4 F, age 45.3y) received whole body hyperthermia + chemotherapy; 6 patients (4 M, 2 F, age 48.8y) received chemotherapy alone.	Radiant heat device	Body temperature range maintained for 60 minutes	Core temperature 41.8–42.2°C	In hyperthermia, a reversible (within 24 hours) decrease in plasma IL-12 and decrease in IFN-γ/IL-10-ratio was seen.
Jimenez et al. 2007	8 subjects: 23±1.5 y, 69.2±2.8 kg, 172±2cm, VO2max 52.0±2.2	1-) neutral temperature seated; 2-) neutral room temperature running at 65% VO2max; 3-) semi-recumbant in heat chamber	1-) 3h; 2-) 2h run, 1h rest; 3-) 2h hot, 1h rest	In 2 + 3: temperature and wind adjusted for 38.5°C at 60 minutes and 39°C at 120 minutes	Leukocytes stimulated with phytohemagglutinin (PHA) or LPS had decreased INFγ and TNFα release in conditions 2 and 3. IL-10 release by LPS increased only with exercise.

Table 1.2B: Effects of heat exposure on cytokines in animal studies

Study	Animal	Heating Method	Duration	Core Temperature	Results
Lin et al. 1994	Male New Zealand rabbits	N/A	Until mean arterial pressure reached 61 mmHg and/or colonic temperature reached 42.8°C	42.8±0.3°C	Heat stroke animals had higher plasma and hypothalamus levels of IL-1β than thermoneutral controls. Heat stroked animals treated with IL-1β receptor antagonist showed improved survival time compared to untreated heat stroke animals.
Chiu et al. 1995	Adult male Sprague-Dawley rats	N/A	Until arterial pressure began to decrease.	42°C	Administering IL-1 receptor antagonist prolonged survival, maintained hypothalamic blood flow, reduced neuronal damage and reduced serotonin accumulation in the brain.
Haveman et al. 1996	Female WAG/Ry rats	Localized hyperthermia of right leg vs. whole body hyperthermia - both by water immersion	Temperature maintained for 60 minutes	Localized: 43°C, 44°C, 45°C or 46°C; whole body: 41.5°C	Whole body hyperthermia led to an increase in plasma IL-1 and IL-6 (IL-1 peaked earlier). IL-1 also increased with sham hyperthermia (anesthesia only). Localized hyperthermia treatment showed no increase in IL-6 and, though IL-1 increased, it increased no more than in the sham.
Lin et al. 1997	Adult male Sprague-Dawley rats	N/A	70 minutes	40.4±0.3°C	Plasma IL-1 levels increased with heat exposure. Treatment with IL-1 receptor antagonist maintained cardiac function and prolonged survival.

Table 1.2B: Effects of heat exposure on cytokines in animal studies

Study	Animal	Heating Method	Duration	Core Temperature	Results
Jiang et al. 1999	Male mice	Water immersion	1-5 hours	37°C, 38°C, 39°C, 39.5°C or 40°C	Plasma LPS-stimulated IL-6, IL-1β and TNFα levels increased with heat exposure.
Ostberg et al. 2000	BALB/c and C57BL/6 female mice	Microisolator cage in a gravity convection oven	6 hours	39.8 ± 0.2°C	No increase in serum IL-1β, IL-6 or TNFα with hyperthermia alone but when low dose LPS was administered prior to heat exposure, IL-6 and TNFα increased with heat exposure. Peritoneal cells of untreated mice were collected, heated and treated with LPS *in vitro*: they showed a decrease in supernatant IL-6 and TNFα.
Ganta et al. 2004	Sprague-Dawley rats with or without splenic denervation	Heat lamp	Temperature maintained for 90 minutes	41°C	Splenic IL-1β and IL-6 levels were elevated in heated rats but not in heated rats with splenic denervation.
Bouchama et al. 2005a	Baboons: 8 heated, 4 "sham heated"	Incubator maintained at 44-47°C	Until designated condition reached.	Moderate heat stroke: 42.5°C. Severe heat stroke: 43.3°C ± 0.1°C	Plasma IL-8, IL-10, and sTNFr I and II increased in hyperthermia, significantly more so with severe heat stroke. TNFα, IL-1β, and IL-4 were not detected at any time in any group.
Bouchama et al. 2005b	Baboons: 8 heated, 4 "sham heated"	Incubator maintained at 44-47°C	Until designated condition reached.	Moderate heat stroke: 42.5°C. Severe heat stroke: 43.3 ± 0.1°C	Increased plasma IL-6 with heat exposure; significantly greater increase with severe heat stroke.

Table 1.2B: Effects of heat exposure on cytokines in animal studies

Study	Animal	Heating Method	Duration	Core Temperature	Results
Chen et al. 2006	8-12 week old mice. Treated with or without neutralizing antibodies for IL-6, TNFα, IL-1β or IFNγ	Environmental chamber set at 38.8°C	6 hours	39.5±0.5 °C	Only mice treated with IL-6 neutralizing antibodies showed impaired leukocyte trafficking in hyperthermia.
Leon et al. 2006	Male C57BL/6J mice	Incubator maintained at 39.5±0.2°C	Until designated temperature reached	42.7°C	Plasma IL-1β, IL-6 and IL-10 were the most increased at the time of the temperature drop during recovery from heat exposure. IL-1α, IL-2, IL-4, IL-12p70, IFN-γ and TNF-α levels did not change at any time in any condition.
Lim et al. 2007	Male Wistar rats	An infrared heating lamp placed 40cm above the rat	Until core temperature was at 42°C for 15 minutes	42°C	Rats treated with turpentine prior to heating to achieve a pre-existing pro-inflammatory state showed a greater increase in plasma IL-6 and IL-1β with heating. Rats pre-treated with dexamethasone showed a lower increase in IL-6 in the heat and no difference in IL-1β compared to non-heated controls.

Table 1.2C: *In vitro* effects of heat exposure on cytokines

Study	Cells used	Heating duration	Temperature	Results
Schmidt and Abdulla. 1988	T-helper cells and peripheral mononuclear cells	4 hours	37°C, 39°C, 41°C or 43°C	LPS stimulated an increased heat shock proteins 70 and 90 release and a reduced IL-1β release at high temperature.
Tomasovic et al. 1989	Murine macrophages, murine EMT-6 mammary adenocarcinoma cells and murine L-929 fibroblast-like cells	1-24 hours	39°C, 40.5°C, 42°C, or 43°C	Timing of LPS treatment affected release of TNFα with heat exposure: administering immediately before or following 1 hour exposure did not trigger TNFα release whereas pre-treating at least 2 hours prior to exposure induced TNFα release. Heating enhanced tumor cell sensitivity to TNFα.
Kappel et al. 1991a	Blood mononuclear cells (healthy subjects)	N/A	37°C or 39°C	When treated with PHA, INFγ release decreased in heat. When treated with LPS, IL-6 and IL-1β release decreased in heat.
Velasco et al. 1991	Fetal astrocytes; human astrocytoma cell lines	N/A	37°C or 40°C	LPS-induced IL-1β and TNFα release was reduced in the heat.
Fouqueray et al. 1992	Rat glomeruli, inflammatory peritoneal macrophages and blood monocytes	20 minutes	37°C, 39°C, 41°C or 43°C	LPS-induced IL-1 and TNFα release was reduced in the heat. The reduction was greater in macrophages and in glomeruli than in monocytes.
Ensor et al. 1994	Human peripheral macrophages	18 hours	37°C, 38.5°C or 40°C	Heat treatment reduced LPS-induced TNFα release. LPS-induced IL-6 release was only mildly reduced and only with high doses of LPS.

Table 1.2C: *In vitro* effects of heat exposure on cytokines

Study	Cells used	Heating duration	Temperature	Results
Ensor et al. 1995	Mouse macrophages	N/A	37°C or 40°C	Heat treatment did not affect LPS-induced TNFα transcription but reduced mRNA stability.
Katschinski et al. 1999	Human myelomonoblastic leukemia cells	1 hour	37°C, 40.5°C, 41°C, 42°C, or 43°C	At 42 and 43°C, a significant increase in apoptosis was seen. This was accompanied by increased TNFα release and TNFα mRNA. Adding TNFα neutralizing antibodies to the culture supernatant largely inhibited apoptosis.
Gnant et al. 2000	Human umbilical vein endothelial cells	90 min or 180 min	37°C, 39°C or 41°C	IL-6 and IL-8 release increased with the combination of hyperthermia and TNFα administration. No difference seen between degrees of heating.
Fairchild et al. 2000	Monocytes and macrophages from umbilical veins of 12 full term infants and 14 healthy adults	24 hours	32°C, 37°C or 40°C	LPS-stimulated release of IL-6, TNFα and IL-1β were similar between 40°C and 37°C after 2 hours incubation at the respective temperatures. However, after 24 hours incubation, the release of the cytokines was lower in heated than in the thermoneutral condition.
Hasday et al. 2001	Human pulmonary artery endothelial cells	24 hours	37°C or 39.5°C	IL-6 release at 37°C was induced by TNFα treatment, an effect mildly suppressed at 39.5°C. IL-8 release was induced by TNFα treatment, to a larger degree at 39.5°C.
Wang et al. 2001	Mouse peritoneal macrophages	15 minutes, 30 minutes, 1 hour or 2 hours	43°C	Heat shock increased LPS-induced IL-10 release and decreased LPS-induced IL-12 release.

Table 1.2C: *In vitro* effects of heat exposure on cytokines

Study	Cells used	Heating duration	Temperature	Results
Shah et al. 2002	Adult and infant micro and macrovascular endothelial cells	8 hours	37°C or 40°C	No changes in IL-1, IL-6, IL-11, IL-12, IL-13 release between conditions.
Shemi et al. 2003	Rat glial cell cultures	1 hour	37°C, 39°C, 42°C or 4°C	No change in LPS-induced TNFα release between warm temperatures and control was seen. However, LPS-induced TNFα release was decreased at 4°C.
Wigmore et al. 2006	Human hepatoma cells	45 minutes	40 or 43°C	Acute phase protein release greater following greater degree of heat exposure. Treating cells with IL-6 prior to heating augmented release of acute phase proteins.

In both patients admitted to hospital with heat shock and in animal heat shock models, levels of inflammatory factors are typically found to be elevated (Bouchama et al. 1991; Bouchama et al. 1993; Bouchama et al. 2000; Bouchama et al. 2005a; Bouchama et al. 2005b; Hammami et al. 1997; Hammami et al. 1998; Hashim et al. 1997; Lin et al. 1994). Animal studies show that the increase in heat-induced pro-inflammatory gene expression is blocked with sympathetic denervation (Ganta et al. 2004), suggesting that the sympathetic nervous system is directly involved in stimulating an inflammatory response to heat stress.

Cancer patients undergoing whole body hyperthermia treatments have increased levels of pro-inflammatory factors post-treatment in comparison to pre-treatment levels (Ahlers et al. 2005; Atanackovic et al. 2002; D'Oleire et al. 1993; Park et al. 1990; Robins et al. 1995). *In vitro* models exposing tumor cells to heat stress show an increased acute phase response in hepatoma cells to the combination of heat stress and the administration of IL-6 (Wigmore et al. 2006), an increased sensitivity to TNFα in heat-stressed adenocarcinoma cells (Tomasovic et al. 1989) and a reduction in heat-induced apoptosis when TNFα is neutralized in myeloplastic leukemia cells (Katschinski et al. 1999).

In addition to pro-inflammatory cytokines, heat exposure triggers the release of heat shock proteins (HSPs), evolutionary conserved proteins normally expressed in the body but which increase rapidly in number in response to cell protein damage, such as during heat stress (Febbraio et al. 2004; Locke. 2002; Pockley. 2003). HSPs stabilize protein structures, act as chaperones in protein folding, help to degrade and remove damaged proteins and regulate rates of transcription and release of inflammatory factors

(Locke. 2002; Pockley. 2003; Yenari et al. 2005). HSPs have been shown to attenuate TNF-α production and release (Moseley. 2002). In skeletal muscle, the release of HSP 72 has been shown to be activated by increased levels of IL-6 (Febbraio et al. 2002). HSPs have also been suggested to be linked to insulin resistance and type 2 diabetes. HSP72 levels have been shown to be decreased in type 2 diabetic patients and to correlate with degree of insulin resistance (Chung et al. 2008; Kurucz et al. 2002). In mice, when HSP72 was overexpressed either in skeletal muscle or globally, increased levels of plasma adiponectin was noted along with protection against diet- or obesity-induced hyperglycemia, hyperinsulinemia, and insulin resistance (Chung et al. 2008).

Cold exposure

Cold exposure can induce hypothermia, a thermoregulatory imbalance where heat loss exceeds heat gain. The inflammatory effects of cold vary between studies. Table 1.3 summarizes the findings of human (1.3A), animal (1.3B) and *in vitro* (1.3C) studies on the effects of cold exposure on inflammatory factors.

Cold exposure appears to consistently increase the release of anti-inflammatory IL-10 in rats and mice (Gundersen et al. 2001; Hildebrand et al. 2005; Huet et al. 2007; Kentner et al. 2002; Lee et al. 2001; Sarcia et al. 2003; Scumpia et al. 2004; Torossian et al. 2004) but the effect remains to be shown in humans. The effect of cold exposure on pro-inflammatory cytokines is somewhat unclear (Fairchild et al. 2005; Hildebrand et al. 2005). In human studies, a variety of inflammatory effects have been seen with cold exposure. Repeated cold water immersion has been shown to raise plasma IL-6 levels (Dugue and Leppanen. 2000; Jansky et al. 1996), though one short post-exercise cold

Table 1.3A: Effects of cold exposure on cytokines in humans

Study	Subjects	Cooling Method	Duration	Core Temperature	Results
Jansky et al. 1996	10 healthy males (mean age 22 years)	Immersion in 14°C water	1 hour, 3 times a week for 6 weeks	N/A	Plasma IL-6 and TNFα tended to increase after multiple immersions while no changes were noted with IL-1β.
Beilin et al. 1998	30 abdominal surgery patients randomized to standard temperature control (hypothermia occurs), or forced body temperature maintenance.	Routine anesthesia care vs. warmed IV fluids and upper body forced warm air cover.	45 minutes pre-operative, intra-operative and 60 minutes post-operative	Routine care core temperature was approximately 1°C lower.	Post-operative peripheral blood monocyte IL-1β and IL-2 release were significantly lower in hypothermia group. Post-operative peripheral blood monocyte TNFα showed a tendency towards being lower in the hypothermia group.
Aibiki et al. 1999a	2 (84 and 87 year old) accidental hypothermia victims.	Accidental hypothermia (1 immersion, 1 non-immersion)	N/A	Below 28°C rectally.	Plasma IL-6 was elevated in both patients on admission and increased further with re-warming. Plasma IL-8 was not elevated on admission but increased on re-warming in the patient with the worse prognosis.
Brenner et al. 1999	7 healthy moderately fit men (mean age 24±1.9 years) pre treated with 1-) exercise, 2-) exercise with thermal clamp (body temperature maintained), 3-) passive heating. This was followed by cold exposure.	Cooling chamber set at 5°C	2 hours	Between 36.5°C (when pretreated with exercise in cool temperature thermal clamp) and 37°C (passive heating).	In cold exposure after all pre-treatments, plasma IL-6 increased. However, in the exercise with temperature clamp pre-treatment, IL-6 levels were already increased prior to cooling.

Table 1.3A: Effects of cold exposure on cytokines in humans

Study	Subjects	Cooling Method	Duration	Core Temperature	Results
Aibiki et al. 1999b	24 severe neurotrauma patients: 10 undergoing normothermic care and 14 undergoing hypothermic care.	Surface cooling	4 days (longer if CT scan warranted)	32-33°C or 36-37°C	Plasma IL-6 levels were more elevated post-trauma in internal jugular venous circulation than in systemic arterial circulation. Post-trauma IL-6 levels decreased more rapidly in hypothermic patients compared to normothermic.
Birdi et al. 1999	45 patients (mean age 64±8 years, 35 males) undergoing elective coronary revascularization.	Cooled systemic perfusate	Duration of surgery	28, 32 and 37°C	Serum IL-8 rose to the same degree in all conditions but IL-8 declined earlier in normothermic than in hypothermic conditions.
Shiozaki et al. 1999	16 patients with severe head injuries	Water-perfused blankets	48 hours	Intracranial temperature 34°C or 37°C	No difference in cerebrospinal fluid TNFα, IL-1β, IL-6, IL-8, and IL-10 between the 2 groups
Hansen et al. 1999	21 patients undergoing elective malignant choroidal melanoma resection: 7 were not resected due to tumor size (controls); during resection, the other 14 underwent chemically-induced hypotension.	Water-perfused blankets in both groups	Duration of surgery	32°C	No changes were seen in serum IL-1β or TNFα. Serum IL-6 increased with the cooling and procedure in both groups (no difference between groups), peaking during rewarming.

Table 1.3A: Effects of cold exposure on cytokines in humans

Study	Subjects	Cooling Method	Duration	Core Temperature	Results
Arons et al. 1999	Intensive care unit patients with known or suspected serious infection: 44 were hypothermic and 409 were febrile.	Infection causing either fever or hypothermia	N/A	Hypothermic: temperature <35.5°C; febrile: temperature ≤38.3°C	Hypothermic patients had higher levels of serum IL-6 and TNFα compared to febrile patients and had reduced survival rates.
Dugue and Leppanen. 2000	11 females (25-52 years, mean age 38.7) and 9 males (19-64 years, mean age 44.0): 5 females and 7 males were regular outdoor winter swimmers.	Sauna heated to 95°C with a relative humidity of 30±50% and cold water swimming	15 minutes in sauna, 2.5 minutes in cold	N/A	Regular winter swimmers had a higher baseline plasma IL-6 level than non-swimmers, but baseline lipopolysaccharide (LPS)-stimulated release of IL-6 and IL-1β was greater in non-swimmers. LPS stimulated IL-6 and IL-1β release from peripheral blood mononuclear cells and whole blood decreased from baseline in non- swimmers and tended to increase from baseline in regular swimmers.
Grünenfelder et al. 2000	Coronary artery bypass grafting with cardiopulmonary bypass patients (25 received normothermic care, 25 received hypothermia care)	Cold perfusate	During surgery	34°C (normothermic) or 24-26°C (hypothermic)	In hypothermic condition, serum IL-6 and IL-8 were significantly higher at 24 hours post-operation and TNFα levels tended to be higher compared to normothermic conditions. Operation duration was longer in hypothermia compared to normothermia. No differences in clinical outcome between groups over 12 day follow-up.

Table 1.3A: Effects of cold exposure on cytokines in humans

Study	Subjects	Cooling Method	Duration	Core Temperature	Results
Rhind et al. 2001	9 men (24.6±3.8yo; $VO_{2\ peak}$ 56.8 ±5.6ml/kg/min)	Over 8 days of exhaustive exercise, 3 "cold wet sessions" consisting of 6x 45 minutes walking, 10 minutes resting in the rain at 5°C	Up to 6 hours per session	day 1: 36.89±0.86; day 8: 36.9±0.78°C	Serum and $CD14^+$ monocyte spontaneous and LPS-stimulated intracellular levels of IL-1β, IL-6, and TNFα increased after exercise, but less IL-1β and TNFα were produced after cold wet conditions following exhausting exercise.
Tokutomi et al. 2004	31 severe head trauma patients treated with hypothermia and 33 not treated with hypothermia	N/A	48 to 72 hours	33°C	Serum CRP levels taken after patient rewarming were higher in the hypothermia group.
Rasmussen et al. 2007	30 (28 M) first time coronary revascularization patients	N/A	During surgery	32°C or 36°C	Plasma IL-6, IL-8, IL-10 response and CRP were independent of temperature.
Peake et al. 2008	10 male endurance-trained cyclists (27±6.7 years) exercising for 90 minutes at hot (32°C) or temperate (18°C) conditions.	Cold water immersion (14°C) or resting at room air following exercise in the heat.	20 minutes	N/A	Cold water immersion during recovery had no detected effect on cytokines or on immune function.
Eggum et al. 2008	30 children under 10 kg body weight undergoing open heart surgery	Cooled perfusate	N/A	32°C or 25°C	Plasma IL-8 increased more in moderate hypothermia group during bypass.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Jiang et al. 1990	Male C57BL/6J mice	Swim tests in 4°C water	1 minute twice daily for 4 days	N/A	Peritoneal macrophages of cold stressed mice had greater levels of secreted and cell-associated IL-1.
Goss et al. 1995	Male Sprague-Dawley rats with traumatic brain injury	Ice bath	4 hours	32°C or 37°C	With neurotrauma, cerebral cortex IL-1β mRNA levels increased. In hypothermia, the increase in IL-1β mRNA was lower than in normothermic rats.
Zhu et al. 1996	Male C57BL/6J mice	Swim tests in 10±2°C water	5 minutes twice daily for 4 days	N/A	Peritoneal macrophages had greater LPS-induced IL-6 release following cold stress than controls.
Dietrich et al. 1999	Wistar rats with forebrain ischemia followed by 1-) normothermia; 2-) IL-10 + normothermia; 3-) hypothermia; 4-) IL-10 + hypothermia	Heating lamp and high speed fan	4 hours	36.5–37°C or 33–34°C	2 months later, though neither hypothermia nor IL-10 treatment alone were protective, improved neuron viability was seen with combined IL-10 and hypothermia treatment.
Patel et al. 2000	Male Sprague Dawley rats with hepatic ischemia	Topical hypothermia (cold water circulated next to liver)	90 minutes	25°C or 37°C	Hypothermic group showed a smaller serum TNFα peak, reduced hepatic necrosis and reduced hepatic neutrophil infiltration.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Angstwurm et al. 2000	Male Wistar rats with bacterial meningitis	Ice packs or heating pad	6 hours (cooled pre meningitis) or 4 hours (cooled post meningitis)	36.5°C, 34.5°C, 32.0°C or 30.5°C	Cerebrospinal fluid TNFα levels were lower at 32.0°C than at thermoneutral conditions.
Sutcliffe et al. 2001	Male C57/Bl6 mice injected with intraperitoneal IL-1β	N/A	1 or 4 hours	32°C or 37°C	Leukocyte rolling and adhesion was stimulated by IL-1β after 1 hour in both temperature groups. However, after 4 hours of hypothermia, leukocyte rolling and adhesion was greatly reduced.
Gundersen et al. 2001	Male Sprague Dawley rats in hemorrhagic shock	Spraying skin with rubbing alcohol	2 hours	32.5–33°C or 37.5±0.5°C	2 hours after hemorrhagic shock, plasma IL-6 increase was significantly lower in hypothermia and TNFα and IL-10 both tended to be decreased.
Lee et al. 2001	Male Sprague Dawley rats	Cooling blanket	8 hours	30°C or 38°C	CD4 and CD8 T-cell intracellular IL-10 increased in the hypothermia group. CD4 IL-2 receptor expression was lower in the hypothermia group.
Yanagawa et al. 2002	Ischemic female C57BL/6 mouse brains	Incubator	24 and 48 hours	33°C or 37°C	IL-6 and IL-1α were released during ischemia. In the cold, the increase in IL-6 was less pronounced and the increase in IL-1α was more pronounced.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Kentner et al. 2002	Rats in hemorrhagic shock	Heating lamp and/or heating pad; electric fan and application of rubbing alcohol to the skin surface during shock and resuscitation.	2 hours	38.0±0.5°C or 34.0±0.5°C	Hypothermic group showed increased 72 hour survival and decreased blood loss. After 60 minutes of resuscitation, serum TNFα levels were significantly higher in the cold condition, IL-10 tended to be higher and IL-6 and IL-1β tended to be lower.
Kinoshita et al. 2002	Sprague-Dawley rats with traumatic brain injury	Cooled air or heating lamp	3 hours	33°C and 39.5°C	No temperature differences in IL-1β mRNA. However, IL-1β protein levels were reduced in the ipsilateral (to injury) cortex, hippocampus and cerebellum in the cold rats compared to other temperatures.
Wu et al. 2003	Male Sprague-Dawley rats in hemorrhagic shock	Topical alcohol and fans	12 hours	34°C or 37.5°C	Increase in serum IL-6 was less during resuscitation with hypothermia and increase in TNFα was greater during resuscitation with hypothermia.
Taniguchi et al. 2003	Male Wistar rats receiving an endotoxin bolus	Heating pad or external cooling	6 hours	36-38°C, 34-35°C or 30-31°C	A reduced peak in plasma IL-6 and TNFα was seen with hypothermia.
Torossian et al. 2003	Male Wistar rats with peritoneal contamination and infection	Ice packs applied to abdomen	1 hour after infection	32°C and 38°C	Hypothermic rats had greater mortality and higher levels of plasma IL-6 compared to normothermic rats. TNFα was not different between groups.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Sarcia et al. 2003	Endotoxemic male Sprague-Dawley rats	Ice pack or heating pad	170 minutes	18- 24°C or 36-38°C	Lung tissue had greater levels of IL-10 and lower levels of IL-1β and IL-6 with hypothermia.
Torossian et al. 2004	Male Wistar rats with peritoneal contamination and infection	Ice packs applied to abdomen	1 hour prior to infection	32°C or 38°C	Rats that were hypothermic prior to infection had increased levels of plasma IL-10 and decreased levels of IL-6 with infection compared to normothermic rats. Infected hypothermic rats also had reduced survival.
Scumpia et al. 2004	Endotoxemic male Sprague-Dawley rats	Ice pack or heating pad	170 minutes	18-24°C or 36-38°C	Heart tissue had greater levels of IL-4 and IL-10 and reduced IL-1β with hypothermia.
Vitarbo et al. 2004	Adult male Sprague-Dawley rats following traumatic brain injury	N/A	3 hours	33°C, 37°C or 39°C	Hypothermia reduced post-traumatic TNFα mRNA expression in the hippocampus. No significant difference in TNFα protein levels was seen in any part of the brain between temperature conditions.
Chu et al. 2005	Sprague-Dawley rats receiving intraperitoneal LPS and either intratrachial LPS or saline solution	Heating or cooling blanket	5 hours	33°C, 37°C or 39°C	Mice receiving intratracheal LPS with hypothermia had lower bronchoalveolar fluid TNFα concentrations.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Hildebrand et al. 2005	81 male C57BL/6 mice following fracture, resuscitation and splint repair of fracture	4 conditions: A: normothermia, B: hypothermia after trauma, C: re-warming after resuscitation and before stabilization and D: hypothermia before trauma. 3 subgroups for B-D according to the degree of hypothermia	N/A Measures taken 3 hours after fracture induced	Subgroup 1: 35–33°C, Subgroup 2: 32.9–30.0°C, Subgroup 3: 29.9–27.0°C	Hypothermia increased survival rates and was associated with lower levels of plasma IL-6 and TNFα and increased IL-10. Rewarming was associated with higher IL-6 and TNFα and lower IL-10 than hypothermia alone.
Truettner et al. 2005	Male Sprague–Dawley rats with traumatic brain injury	Cooled air and heating lamps	3 hours	33°C or 37°C	Post traumatic increase in brain IL-1β gene expressed tended to be less in hypothermia than in normothermia. IL-2 and IL-6 gene expression increased post-trauma but no temperature difference was seen.
Davis et al. 2005	8 healthy adult horses following submaximal exercise breathing either cold (-5°C) or temperate air (25°C)	Air chiller connected to face mask	15 minutes	N/A	Bronchoalveolar lavage fluid levels of IL-6, IL-2, IL-4, IL-5 and IL-10 mRNA increased significantly following exercise in the cold. No significant difference has seen in IL-1α, IL-1 β, IL-8, TNFα and IFNγ mRNA.

Table 1.3B: Effects of cold exposure on cytokines in animal studies

Study	Animal	Cooling Method	Duration	Core Temperature	Results
Ganta et al. 2006	F344 rats with either splenic innervation intact or denervated	Heating blanket and ice packs	80 minutes	30°C or 38°C	Gene-array analysis of spleen tissue showed increased expression in hypothermia of IL-6 and IL-1β in both splenic intact and denervated rats.
Hangalapura et al. 2006	Chickens from lines selected for high or low antibody production	Cooling chambers set at 10°C or 21.7±1.9°C	42 days	N/A	Peripheral blood leukocyte mRNA expression of IL-1β, IL-6, IL-12β and IL-4 were increased following cold stress in both groups.
Huet et al. 2007	Male Sprague–Dawley rats given intraperitoneal LPS	Cold blanket	2 hours	32°C or 38°C	IL-10 was significantly greater in hypothermia group. ΔIL-10/ΔTNFα ratio was significantly greater in hypothermia compared to normothermia.

Table 1.3C: *In vitro* effects of cold exposure on cytokines

Study	Cells used	Cooling duration	Temperature	Results
Fairchild et al. 2000	Monocytes and macrophages from venous blood from umbilical veins of 12 full term infants and 14 healthy adults	24 hours	32°C, 37°C, or 40°C	Cells were incubated with LPS: LPS-stimulated release of IL-6, TNFα and IL-1β were delayed at 32°C but overall increased and prolonged.
Sutcliffe et al. 2001	Human cerebral endothelial cells incubated with IL-1β	4 hours	37°C or 32°C	Human cerebral endothelial cells showed a reduced NFκβ response to IL-1β in cold conditions. Human cerebral endothelial cell IL-1β-stimulated IL-1β and IL-8 mRNA production were reduced in the cold, as was IL-8 release into the medium.
Russwurm et al. 2002	Human peripheral blood mononuclear cells stimulated by phytohemagglutinin (PHA), LPS or TNFα	4 hours	32°C or 34°C	All cold conditions and stimulants showed decreased IL-2 release. IL-6, IL-10 and TNFα release with PHA was reduced in the cold. IL-10 release was mildly reduced with TNFα stimulation in the cold. Without any stimulation, TNFα release was mildly increased in the cold.
Kimura et al. 2002	Human peripheral blood mononuclear cells	1 hour	33°C or 37°C	Cells incubated with LPS in the cold showed delayed NFκβ activation as well as delayed release of IL-1β, IL-6 and TNFα.
Maekawa et al. 2002	Microglial cells isolated from primary cultures of rat brains.	N/A	30°C, 33°C or 37°C	LPS-induced IL-6 release was less in cells incubated at lower temperatures for 48 hours following stimulation. LPS-induced TNFα release was only reduced for 6 hours after stimulation.

Table 1.3C: *In vitro* effects of cold exposure on cytokines

Study	Cells used	Cooling duration	Temperature	Results
Matsui et al. 2004	Peripheral blood mononuclear cells isolated from 12 healthy human subjects	24 hours	33°C or 37°C	PHA-induced IL-10 and INFγ release were reduced in the cold. No temperature-related difference was noted for IL-6, IL-8 or TNFα. The ratio of IL-10 to pro-inflammatory cytokine was significantly reduced in the cold.
Fairchild et al. 2004	Human THP-1 monocyte cell line	15 minutes to 4 hours	32°C	LPS-induced IL-1β and TNFα release is delayed but increased in the cold. These results were also seen when LPS was replaced by opsonized zymosan or toxic shock syndrome toxin 1. Stability of TNFα mRNA was not changed in cold exposure but mRNA transcription was increased.
Fairchild et al. 2005	Human promonocytic THP-1 cells	N/A	28°C or 32°C	TNFα release in response to LPS is delayed both in and following cold exposure, but the peak TNFα release is greater.
Matsui et al. 2006	Human monocytes from 10 healthy 20 to 40 year old subjects	48 hours	33°C or 37°C	LPS-stimulated IL-1β, IL-6, IL-12p70, and TNFα release are increased with hypothermia. No significant temperature-related difference is seen in IL-8 or IL-10 release. At 33°C, IL-12p70/IL-10 and TNFα]/IL-10 ratios were significantly increased.
Noda et al. 2008	Human umbilical vein endothelial cells	5, 12 or 24 hours	30°C or 37°C	IL-8 mRNA and protein levels are lower with cold exposure. However, when culture is treated with LPS and glucose, there is an increase in IL-8 regardless of temperature.

water immersion session following exercise did not have any effect on pro-inflammatory cytokines (Peake et al. 2008). Accidental hypothermia patients have been shown to have elevated plasma IL-6 on hospital admission (Aibiki et al. 1999a). Intraoperative hypothermia has been associated with smaller elevations in pro-inflammatory cytokines (Beilin et al. 1998), longer elevations of pro-inflammatory cytokines (Birdi et al. 1999), no temperature effect on cytokines (Rasmussen et al. 2007) or greater elevations of pro-inflammatory cytokines (Eggum et al. 2008; Grünenfelder et al. 2000) compared to thermoneutral intra-operative conditions. Hypothermic treatment of head injuries has been associated with either decreased (Aibiki et al. 1999b) or equivocal (Shiozaki et al. 1999) levels of pro-inflammatory cytokines. In animal models, cooling with either water immersion or a cooling chamber increased pro-inflammatory cytokine release when isolated white blood cells were stimulated with lipopolysaccharide (LPS) (Hangalapura et al. 2006; Jiang et al. 1990; Zhu et al. 1996). In animal models of trauma, endotoxemia or infection, cooling has consistently reduced the increase in pro-inflammatory factors or favored the balance of anti-inflammatory to pro-inflammatory factors (Angstwurm et al. 2000; Chu et al. 2005; Gundersen et al. 2001; Hildebrand et al. 2005; Huet et al. 2007; Kinoshita et al. 2002; Patel et al. 2000; Sarcia et al. 2003; Scumpia et al. 2004; Taniguchi et al. 2003; Torossian et al. 2003; Torossian et al. 2004; Truettner et al. 2005). One possible exception is that in 2 of the 3 animal models of hemorrhagic shock, IL-6 levels decreased but TNFα levels increased with cold exposure (Kentner et al. 2002; Wu et al. 2003). Rewarming following hypothermic therapy appears to be related to elevations in pro-inflammatory cytokines (Hansen et al. 1999; Hildebrand et al. 2005; Tokutomi et al. 2004) in both human and animal models. *In vitro* work has suggested that the rate of

inflammatory response to injury in the cold is lagged in comparison to inflammatory response at a neutral temperature but that the response is increased in intensity and prolonged in duration (Fairchild et al. 2004). Hypothermia has, however, also been shown to be protective against cell necrosis during ischemic conditions and to counter the pro-inflammatory events that accompany cell necrosis (Qing et al. 2002) and has been suggested to reduce apoptosis (programmed cell death) (Zhao et al. 2005). Cold exposure apparently has both a potential for inflammatory-related tissue damage as well as for stimulating anti-inflammatory and cytoprotective mechanisms (Fairchild et al. 2004; Fairchild et al. 2005).

There are many possible reasons for the varied inflammatory responses to a decrease in body temperature seen in the reviewed research. These reasons include the degree of cooling, the presence of other co-existing stresses such as water immersion or anesthesia commonly used in animal cold stress studies, co-existent medical problems such as cardiac infarction, head trauma or infection often seen in both clinical studies of cold exposure as well as in animal clinical models and the presence of substances that stimulate inflammatory response such as LPS or phytohemagglutinin (PHA).

Cold has also been shown to affect adipokines. Multiple studies have shown that leptin decreases with cold exposure (Ricci et al. 2000; Zeyl et al. 2004). The effect of cold exposure on circulating adiponectin has been explored in rodents with inconsistent results with one study showing an increase in circulating adiponectin (Yoda et al. 2001), one showing a decrease (Imai et al. 2006) and one showing no effect (Puerta et al. 2002). In a preliminary study from our research team, anti-inflammatory adiponectin levels have been shown to increase significantly in young healthy men during cold exposure, which

is the first time that any acute stress has been shown to affect adiponectin levels (Haman and Imbeault. 2005; Imbeault and Haman. 2007).

Summary

In summary, inflammation is related to fat mass and affects the progression of numerous chronic diseases. The cytokines, IL-6 and TNFα are two of the major markers of inflammation and are some of the most documented inflammatory markers in the current literature. Adiponectin is a factor released by adipose tissue, though indirectly proportional to fat mass, and is protective against both insulin resistance and inflammation. Acute stresses, including environmental stresses such as heat and cold exposure, have the potential to modify inflammation.

Rationale for thesis

Based on the review of literature, the following knowledge gaps and ideas about these knowledge gaps were identified:

1-) With heat stress:

Most heat exposure studies that have examined immune or inflammatory response with heat exposure have assessed either active hyperthermia models or whole body hyperthermia therapy in cancer patients and show an increase in pro-inflammatory activity in response to heat stress (Ahlers et al. 2005; Atanackovic et al. 2002; D'Oleire et al. 1993; Park et al. 1990; Robins et al. 1995; Walsh and Whitham. 2006). The series of studies assessing heat shock in pilgrims to Mecca is a mixed model of active and passive hyperthermia in that body temperature is influenced by both high environmental temperature and a high degree of physical exertion; these studies consistently show

increased levels of inflammatory factors in heat shock patients (Bouchama et al. 1991; Bouchama et al. 1993; Hammami et al. 1997; Hammami et al. 1998; Hashim et al. 1997). To our knowledge, the only study looking at heat-related effects on adiponectin was one rodent study has shown that the over-expression of HSP72, a factor released during heat exposure, increases circulating levels of adiponectin (Chung 2008). Based on the reviewed evidence, we expect that, despite the lower degree of heat stress used in this study compared to previous work, plasma concentrations of IL-6, TNFα and adiponectin will all increase with heat stress.

2-) With cold stress:

Though not yet clear in humans, animal studies have shown an increased anti-inflammatory response to cold exposure (Gundersen et al. 2001; Hildebrand et al. 2005; Huet et al. 2007; Kentner et al. 2002; Lee et al. 2001; Sarcia et al. 2003; Scumpia et al. 2004; Torossian et al. 2004). The effect of acute cold exposure has mainly been investigated in conjunction with other stresses, be they exercise, injury or illness but these studies do suggest that cold exposure modifies inflammatory response and, for the most part, reduces the release of pro-inflammatory cytokines (Aibiki et al. 1999b; Angstwurm et al. 2000; Beilin et al. 1998; Chu et al. 2005; Gundersen et al. 2001; Hildebrand et al. 2005; Huet et al. 2007; Kinoshita et al. 2002; Patel et al. 2000; Sarcia et al. 2003; Scumpia et al. 2004; Taniguchi et al. 2003; Torossian et al. 2003; Torossian et al. 2004; Truettner et al. 2005). Previous work during cold exposure has shown that adiponectin levels can increase in humans by 25 to 70% during cold exposure, depending on baseline values (Haman and Imbeault. 2005; Imbeault and Haman. 2007). Based on these

observations, we anticipate that, during cold exposure, circulating IL-6 and TNFα will decrease and plasma adiponectin will increase.

Thesis objectives and hypotheses

Objectives

The goal of this thesis is to use temperature stress as a model to examine the inflammatory responses to acute stress.

In order to assess the effects of temperature stress on inflammatory factors, a series of experiments was used. The specific objectives of these experiments were:

1-) To quantify the effects of different intensities and durations of heat exposure on changes in circulating IL-6, TNFα and adiponectin (Experiments 1 and 2).

2-) To determine the effects of cold exposure on changes in circulating IL-6 and TNFα using blood samples collected previously in men exposed to 5°C for 120 minutes. These measures will be related to previously quantified changes in circulating adiponectin (Experiment 3).

This thesis consists of a series of three experiments: consisting of two different intensities and durations of heat exposure as well as a cold exposure experiment. The first study performed was part of an industry-sponsored project (Med Eng, Ottawa, Canada) testing the effectiveness of personal cooling systems on military personnel during heat exposure (Experiment 1). A control session (heat exposure without personal cooling system) was done to look at the effect of heat exposure over 3 hours with pre and post heat-exposure measurements of inflammatory factors. In order to compare heat exposure to cold exposure, this experiment was repeated (Experiment 2) to match the protocol (in terms of durations and data collection times) used in a previously conducted

cold exposure study (Experiment 3, original study by Dépeault, I – thesis in progress) assessing the effects of 120 minutes of cold exposure on fuel selection and metabolism. New analyses from plasma samples taken from Experiment 3 were performed by the author for this thesis (IL-6 and TNFα). Previously measured metabolic data and plasma adiponectin measurements from Experiment 3 are used in this thesis for the purpose of comparing this data to original data from Experiments 1 and 2. Figure 1.2 summarizes the series of experiments that make up this thesis.

Hypotheses

1-) With heat stress: plasma IL-6, TNFα and adiponectin will all increase. A larger increase will be seen with greater heating intensity or duration.

2-) With cold stress: circulating IL-6 and TNFα will decrease while plasma adiponectin concentration will increase.

The rest of the thesis will be laid out as follows: The following chapter will discuss the methodology used for this study. Chapter 3 will look at the results of the study which will be further analyzed in Chapter 4, both looking at heat and cold exposure separately as well as comparing and contrasting the two conditions. Finally, Chapter 5 will consist of a brief discussion of future directions and concluding remarks.

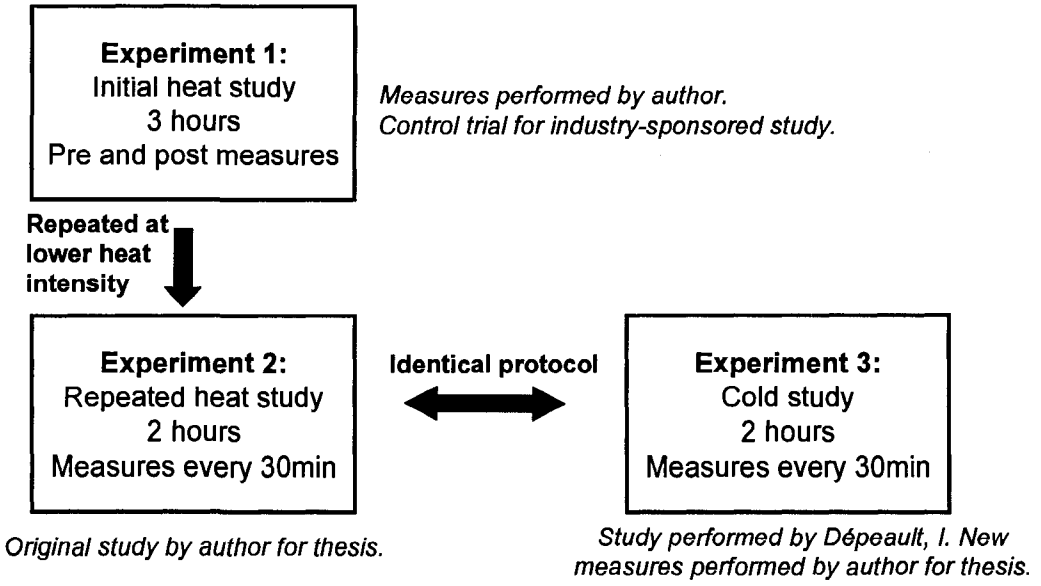

Figure 1.2: Overview of trials comprising thesis project.

Chapter 2 – Methodology

This thesis consists of a series of three experiments. All components of the study have been approved by the University of Ottawa Ethics Board (file H 09-05-03) and subjects for all studies were volunteers from whom informed consent was obtained. An overview of the three experiments can be seen in Figure 1.2.

Experiment 1 - Initial heat exposure trial

The experiment was the control trial (without cooling system) of an industry-sponsored project (Med Eng, Ottawa, Canada) testing the effectiveness of personal cooling systems on military personnel during heat exposure. Six young healthy male volunteers (mean age 27±5, weight 69.9±3.1kg, height 170.7±1.3cm) recruited from a university population were dressed in military attire (helmet, shirt, OTV Kevlar vest, pants and boots). They were seated for a 20 minute rest at room temperature before being transferred into a heat chamber set at 42°C and 30% humidity for 180 minutes. Subjects remained seated for the whole process and were given 1L of water to drink during the heat exposure to compensate for fluid losses. Expired gases were measured throughout the resting and heat exposure periods (Moxus Metabolic System, AEI Technologies, Pittsburg, PE). Core body temperatures were monitored throughout the study for participant safety, using the Vital Sense Jonah™ Ingestible Core Temperature Capsule (Mini Mitter Co, Bend, OR). If the core temperature of any participant were to reach 39.5°C at any point in the study, they were to be removed from the heat. No participant reached that core temperature and all participants completed the study.

Blood samples were collected after the 20 minute rest at room temperature and then immediately following the heat exposure. Blood samples were placed on ice and centrifuged within 10 minutes at 3000 rpm for 8 minutes at 4°C to separate the plasma

from the red blood cells. The plasma was then immediately stored at -80°C until further measurements were performed.

Plasma IL-6, TNFα and adiponectin levels were measured using a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winooski, Vt.), using KC4 microplate data analysis software (Bio-Tek Instruments Inc., Highland Park, Winooski, Vt.). Commercial enzyme-linked immunosorbent assay (ELISA) kits (R and D Systems, Minneapolis, MN for IL-6 and TNFα; ALPCO Diagnostics, Salem, NH for adiponectin) were used. All samples were analyzed in duplicate and samples at all points for the same subject were analyzed in the same batch, except for one subject from whom only a single sample was analyzed for TNFα due to insufficient plasma.

IL-6 and TNFα have diurnal patterns. Both factors have morning troughs which should correspond with the time period during which measurements occurred (Petrovsky and Harrison. 1998). However, in order to account for any variability of cytokine levels due to circadian rhythms during the 3 hours of measurement, an additional arm of the study was used as a temperature control. Three young healthy male volunteers were recruited from a university population. These subjects were seated at room temperature for 180 minutes. Blood samples were obtained at the beginning and at the end of the 180 minute trial. Plasma IL-6 and TNFα were measured as described above. It should be noted that plasma adiponectin was not measured in this thermoneutral condition since it is well established that this adipose-tissue derived protein is not affected by circadian variation (Hotta et al. 2000; Peake et al. 2003).

Experiments 2 and 3 - Comparative heat and cold stress studies

Subjects

Twelve healthy male participants were recruited from the university population. Exclusion criteria included any known cardiac disease, pulmonary disease or diabetes. Six participants took part in the cold exposure arm of the study and six participants took part in the heat exposure arm of the study. Subject characteristics from both arms of the study are shown in Table 2.1.

Preparations prior to testing

Participants were instructed to arrive at the laboratory at 8 am on the day of the trial and were instructed to have fasted as of 10 pm the night before with no consumption of caffeine or alcohol in the past 24 hours. Participants were asked to refrain from intense physical activity on the day of the study. On the morning of the trial, heat exposure subjects were given a questionnaire modified from the 24 Hour Physical Activity Recall Questionnaire of the Kuopio Ischemic Heart Disease Study (KIHD) (Salonen and Lakka. 1987). The format of the KIHD questionnaire was kept intact but the grading of activities was modified using the scale from the Bouchard 3-Day Physical Activity Questionnaire (Bouchard et al. 1983) to include activities that were more relevant to the subject population. A copy of the questionnaire distributed to subjects is found in Appendix A. The 24 hour physical activity levels for these subjects are found in Appendix B.

Height, body weight and body composition were measured on the morning of the trial. Body weight was measured using a standard scale after voiding with the subjects

Table 2.1 : Subject characteristics of the cold stress and heat stress study arms.

Characteristic	Cold stress mean (SE)	Heat stress mean (SE)	P-value
Age	24 (2)	24 (2)	NS
Weight (kg)	71.7 (2.2)	82.1 (2.7)	<0.05
Height (cm)	177.7 (1.9)	176.3 (3.1)	NS
Percent fat mass	12.1 (1.4)	17.9 (2.7)	NS
Fat mass (kg)	8.6 (1.4)	14.3 (2.3)	<0.05
Lean mass (kg)	60.3 (1.2)	64.3 (1.9)	NS
VO2 peak (ml/kg/min)	57.7 (3.0)	51.9 (3.8)	NS

dressed in a t-shirt and underwear. Height was measured using a measuring tape with the participant standing in bare feet with their feet together and their heels, buttocks, back, and head against the wall, following a normal inspiration. Body composition was measured using dual-energy x-ray absorptiometry (DEXA) using a LUNAR Prodigy (GE Healthcare, Waukesha, Winsconsin) and analyzed with CLS Clinical Reporting Software (Aymes Medical Software, Inc., Aurora, Ontario).

Temperature stress

Subjects were equipped with a liquid conditioning suit that covered the head, torso and upper and lower extremities (Med Eng, Ottawa, ON). After sitting at room temperature for 60 minutes to establish a stable baseline, water set at either 48°C (Experiment 2 - heat stress condition) or 4°C (Experiment 3 - cold stress condition) was pumped through the suit for 120 minutes. Subjects remained semi-recumbent for the whole baseline and temperature stress period. To compensate for water loss during heat exposure, subjects were given 1230ml of water to consume during the heat session.

Temperature and metabolic data measurements

During the cold stress study, core temperature was monitored using a tympanic temperature probe (Mon-a-therm Tympanic, Mallinckrodt Medical, St. Louis, MO). This was done by placing a thermo couple in direct contact with the tympanic membrane. The position of the probe was then secured by taping the thermo couple wire to the external ear. Though not as accurate as more invasive measures of core temperature (Deschamps et al. 1992; Roth et al. 1996), this method was felt to be able to provide us

with information regarding changes in body temperature. It was felt that more invasive measures were not warranted.

Due to concern regarding the possibility of heat-related illness during the heat stress condition (Barrow and Clark. 1998), a more accurate measure of core temperature was warranted. For participant safety, core body temperatures were monitored throughout the heat stress study using the Vital Sense Jonah™ Ingestible Core Temperature Capsule (Mini Mitter Co, Bend, OR). If the core temperature of any participant were to reach 39.5°C at any point in the study, they were to be removed from the heat. No participant reached that core temperature and all participants completed the study. A tympanic temperature probe was used in 3 heat exposure subjects due to concerns during the trial that the reader was not picking up signals from the capsule.

Expired gases were measured throughout the resting and temperature stress periods using an indirect calorimetric metabolic cart (Moxus Metabolic System, AEI Technologies, Pittsburg, PE). Heart rate was measured throughout the trials using a continuous heart rate monitor (Polar Electro, Kempele, Finland).

Blood sampling and biochemistry

Blood samples were collected through an indwelling catheter (18G, 32mm, Medical Inc., Arlington, TX) placed in an antecubital vein at the start of and halfway through the room temperature rest period and then at times 0, 30, 60, 90 and 120 minutes during the temperature stress session in Vacutainer tubes (BD, Franklin Lakes, NJ) with ethylene diamine tetraacetic acid (EDTA). Figure 2.2 demonstrates the timing of the blood draws. The hematocrit was measured before and after the heat exposure in order to assess for dehydration, which would affect biochemical measurements (Eddy and et al.

1976). Blood samples were placed on ice and centrifuged within 10 minutes at 3000 rpm for 8 minutes at 4°C to separate the plasma from the red blood cells. The plasma was then immediately stored at -80°C until further measurements were performed.

Biochemical measures were taken from plasma samples using ELISA methods and analyzed on a Synergy HT Series Multi-Detection Reader (Bio-Tek Instruments Inc., Highland Park, Winooski, Vt.), using KC4 microplate data analysis software (Bio-Tek Instruments Inc., Highland Park, Winooski, Vt.). Commercial kits were used to measure plasma adiponectin (Linco Research, St Charles MO), IL-6 and TNFα (R and D Systems, Minneapolis, MN). All samples were analyzed in duplicate and samples at all points for the same subject were analyzed in the same batch.

Statistical analyses

Independent T tests were performed on baseline characteristics of subjects to verify any differences between groups. Continuous measurements were averaged during the resting period and for every 15 minute period during temperature stress. Paired T-tests were performed to compare the pre and post values of plasma factors in both the initial heat exposure study and in the thermoneutral control study. Repeated measures ANOVA was used to assess differences of biochemical and metabolic measures over time. Any significant repeated measure ANOVAs were further assessed using Dunnett's method to determine between which points statistical significance occurred. Results are expressed as the mean and standard error of the mean (SEM). Measurements from the two heat trials were combined and Pearson's two-way correlations were run to examine any relationship between changes in biochemical markers and changes in core body temperature. SPSS v.16.0 software was used for all statistical analyses.

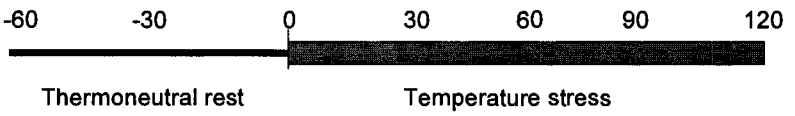

Figure 2.2: Time line for blood sampling during temperature stress trials.

Chapter 3 – Results

Experiment 1 - Initial heat exposure trial

During the first heat exposure study, consisting of 3 hours in a heated chamber set at 42°C, core body temperature increased significantly during the heat exposure trial: from 36.9±0.1°C to a maximum temperature of 38.0±0.1°C, $p<0.05$. Figure 3.1 shows the changes in oxygen consumption, ventilation (VE) and heart rate over time during the baseline rest period and the three hours of heat exposure. Oxygen consumption increased from 255.0±18.3 ml/min to 327.6±26.4 ml/min (significant effect of time, $p<0.01$). VE increased from 9.2±0.6 L/min to 11.8±1.0 L/min (significant effect of time, $p<0.01$). Heart rate increased from 74±4bpm to 102±5bpm (significant effect of time, $p=0.001$).

Figure 3.2 shows plasma IL-6, TNFα, and total adiponectin values before and following heat exposure and the 3 hour thermoneutral condition. During heat exposure, plasma IL-6 increased significantly from baseline, from 0.70±0.09pg/ml to 3.39±0.89pg/ml ($p<0.05$). Plasma TNFα and adiponectin showed a small non-significant change from baseline: changing from 1.23±0.22pg/ml to 1.31±0.19pg/ml (p=NS) and from 5.18±0.58μg/ml to 5.72±0.31μg/ml (p=NS), respectively, following 3 hours of heat exposure. No significant change in plasma IL-6 or TNFα was seen between the two time points in thermoneutral conditions.

Experiment 2 - Heat stress

During the second heat stress study consisting of two hours of heat exposure, core body temperature increased from 36.6±0.2°C to 37.0±0.2°C ($p=0.06$). Figure 3.3 shows the changes in oxygen consumption, VE and heart rate over time during the baseline rest period and the two hours of heat exposure. A small but significant rise in oxygen

consumption was observed (from 291.18±9.45 ml/min to 337.63±7.62 ml/min, significant effect of time, $p<0.05$). VE did not change significantly (from 9.18±0.37L/min to 10.93±0.91L/min, no significant effect of time). Heart rate increased from 60.5±5.6 bpm to 81.5±7.8 bpm, significant effect of time, $p<0.05$.

Figure 3.4 shows changes in plasma IL-6, TNFα and adiponectin over time during rest and 2 hours of heat exposure. During this trial, fasting plasma IL-6, TNFα and adiponectin did not show any significant change from baseline values.

In order to take a closer look at the potential effect that differences in core temperatures from the two trials had on inflammation, further analysis was done pooling the data from Experiments 1 and 2. When data from Experiments 1 and 2 were pooled together and absolute change in core temperature and absolute change in plasma IL-6 were plotted against one another, as shown in Figure 3.5, a significant positive Pearson's correlation was found between the absolute change in core body temperature and the absolute in plasma IL-6 concentration ($r=0.684$, $p=0.014$). Similarly, as shown in Figure 3.6, a positive Pearson's correlation between changes in body temperature and changes in plasma adiponectin was found ($r=0.616$, $p=0.033$). No such correlation was seen been change in core temperature and change in plasma TNFα.

Experiment 3 - Cold stress

During the two hours of cold exposure, core temperature did not change significantly from 35.9±0.1°C at baseline to 35.7±0.2°C. Figure 3.7 shows the changes in oxygen consumption, VE and heart rate over time during the baseline rest period and the two hours of cold exposure. Oxygen consumption increased from 307.0 ±14.8ml/min to 768.8±46.2ml/min (significant effect of time, $p<0.001$). VE increased from

9.9±0.5L/min to 20.7±1.4L/min (significant effect of time, $p<0.001$). Heart rate increased from 59.6±2.2bpm to 72.1±4.7bpm (significant effect of time, $p<0.01$).

Figure 3.8 shows changes in plasma IL-6, TNFα and adiponectin over time during rest and 2 hours of cold exposure. Plasma IL-6 tended to increase from 0.89±0.12pg/ml to 1.79±0.69pg/ml (effect of time, $p=0.065$) and plasma TNFα had a small non-significant change from 1.46±0.51pg/ml to 1.80±0.67pg/ml. Plasma total adiponectin increased from 12.18±1.57μg/ml to a maximum level of 14.51±2.50 μg/ml, but this fell short of statistical significance (effect of time, $p=0.09$).

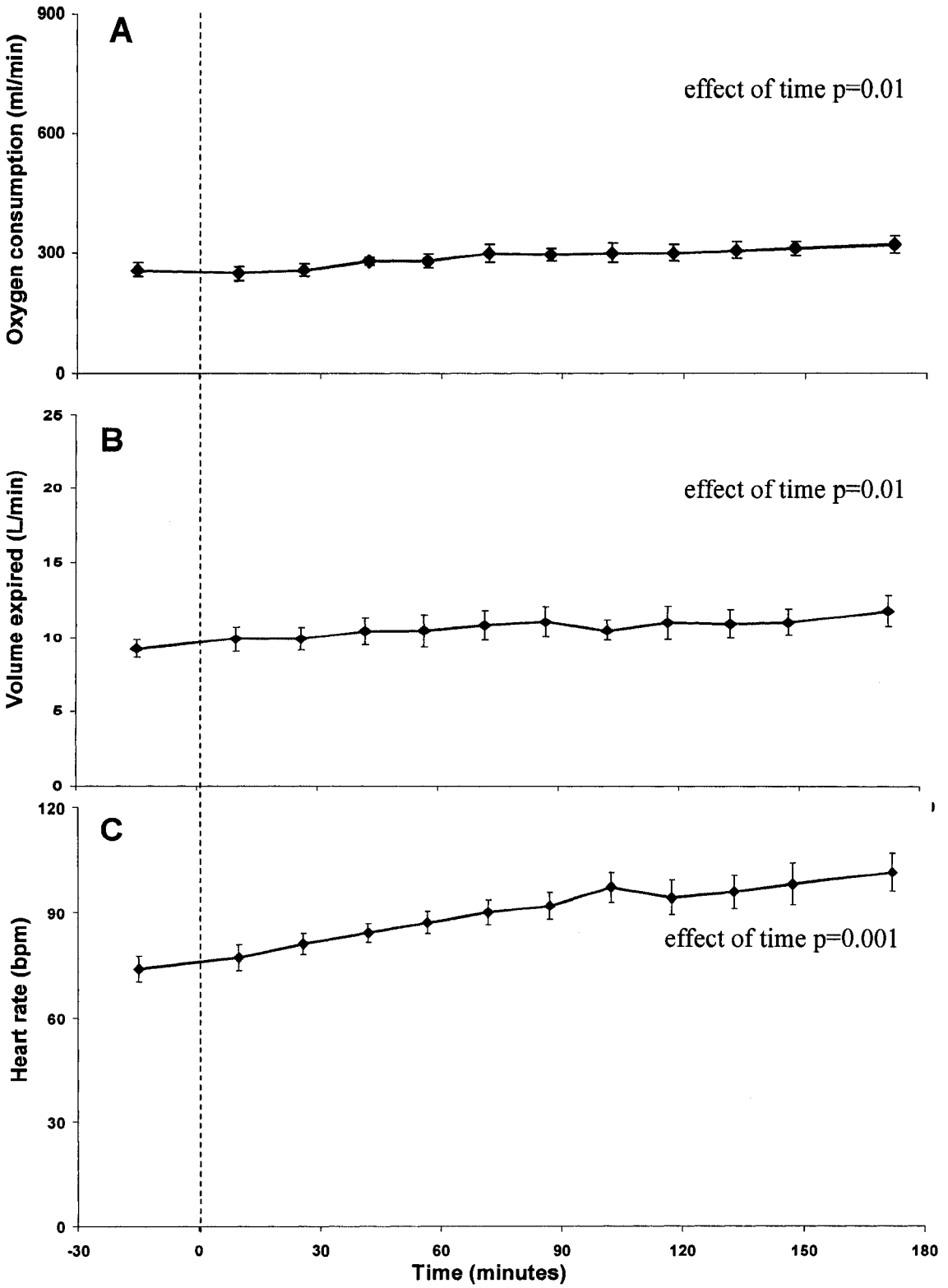

Figure 3.1: Changes in total oxygen consumption (A), VE (B) and heart rate (C) over time during 3 hours of heat exposure in heated chamber (Experiment 1). Statistically significant effect of time: energy expenditure (p=0.01), VE (p=0.01), heart rate (p=0.001).

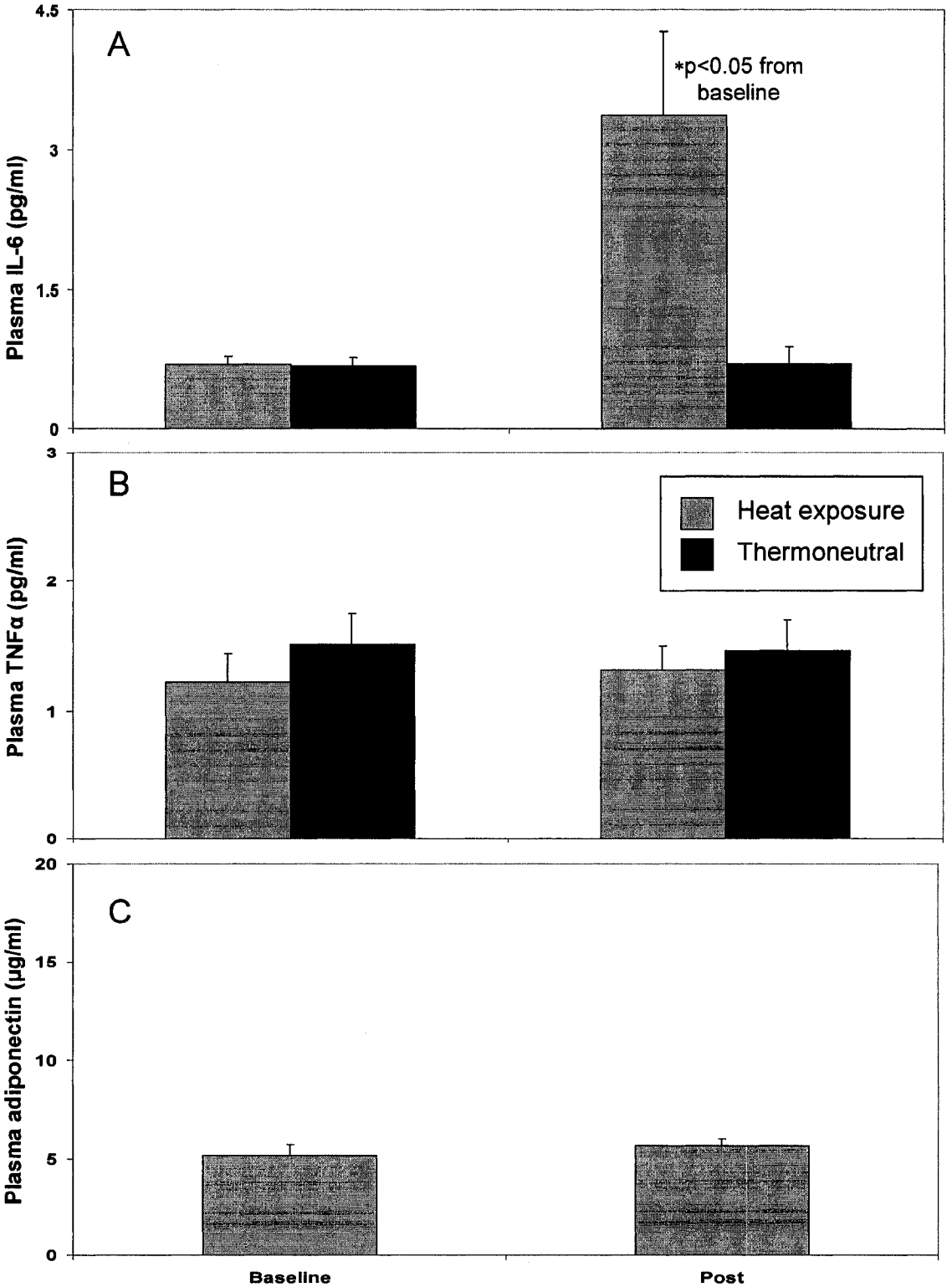

Figure 3.2: Changes in plasma IL-6 (A), TNFα (B) and adiponectin (C) from baseline after 3 hours of heat exposure in heated chamber in 6 subjects (grey bars) and after 3 hours of thermoneutral temperature in 3 subjects (black bars) (Experiment 1). Statistically significant effect of time: IL-6 ($p<0.05$). Thermoneutral measurements of plasma adiponectin were not done as it has been previously established that plasma adiponectin does not follow a circadian pattern.

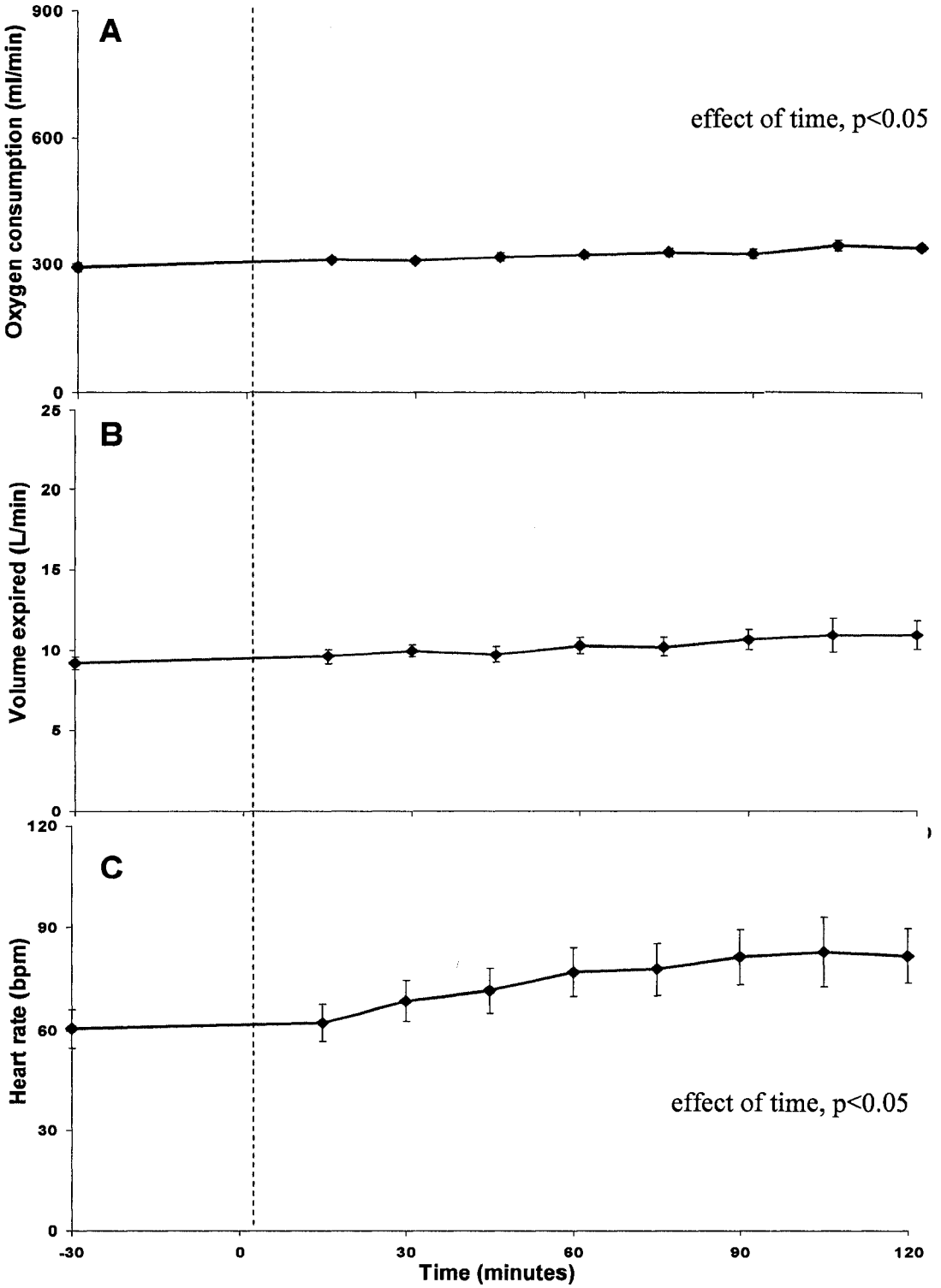

Figure 3.3: Changes in oxygen consumption (A), VE (B) and heart rate (C) over time during 2 hours of heat exposure with liquid-conditioned suit (Experiment 2). Significant effect of time: energy expenditure ($p<0.05$), heart rate ($p<0.05$).

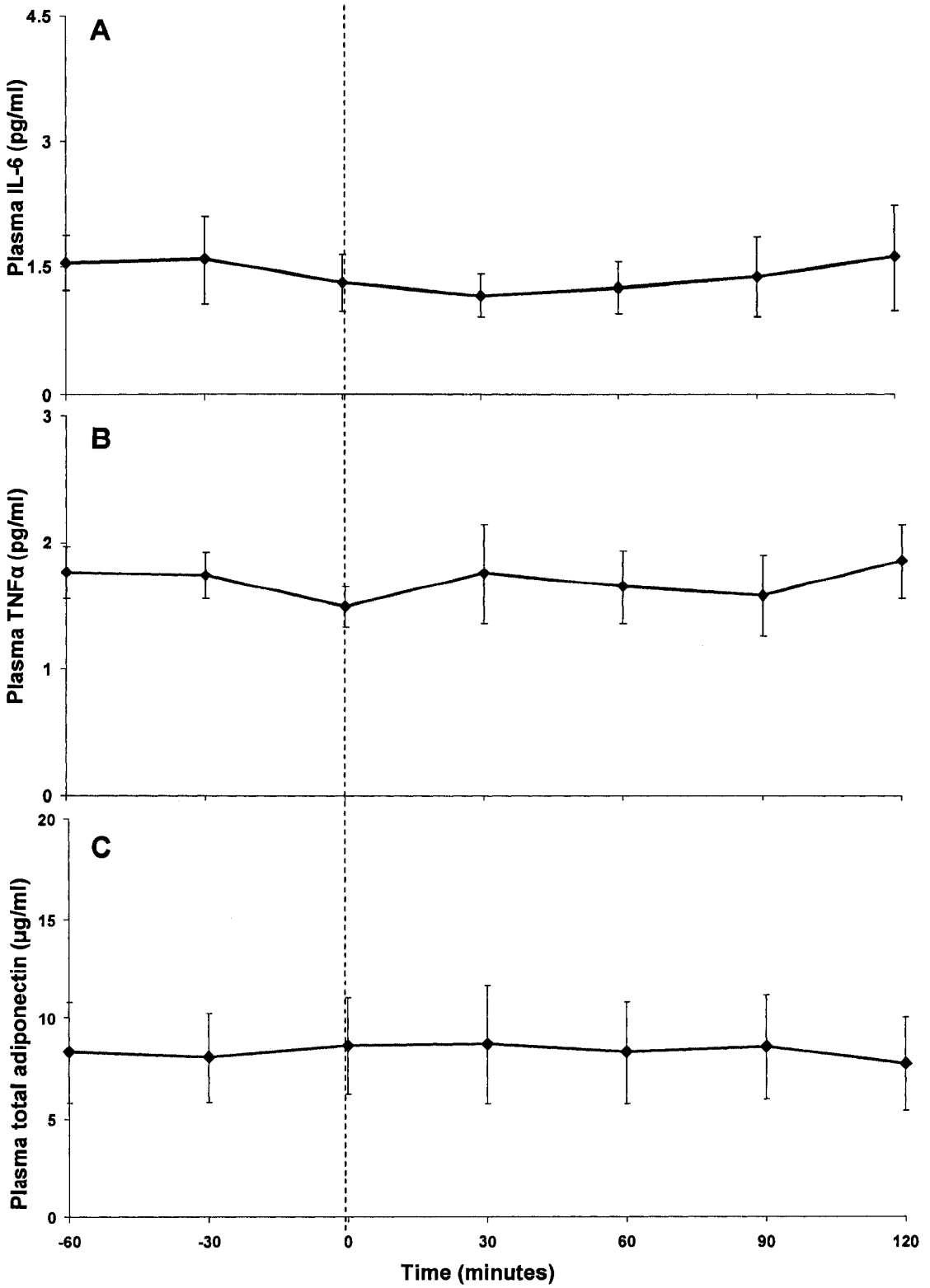

Figure 3.4: Changes in plasma IL-6 (A), TNFα (B) and total adiponectin (C) over time during 2 hours of heat exposure with liquid-conditioned suit (Experiment 2).

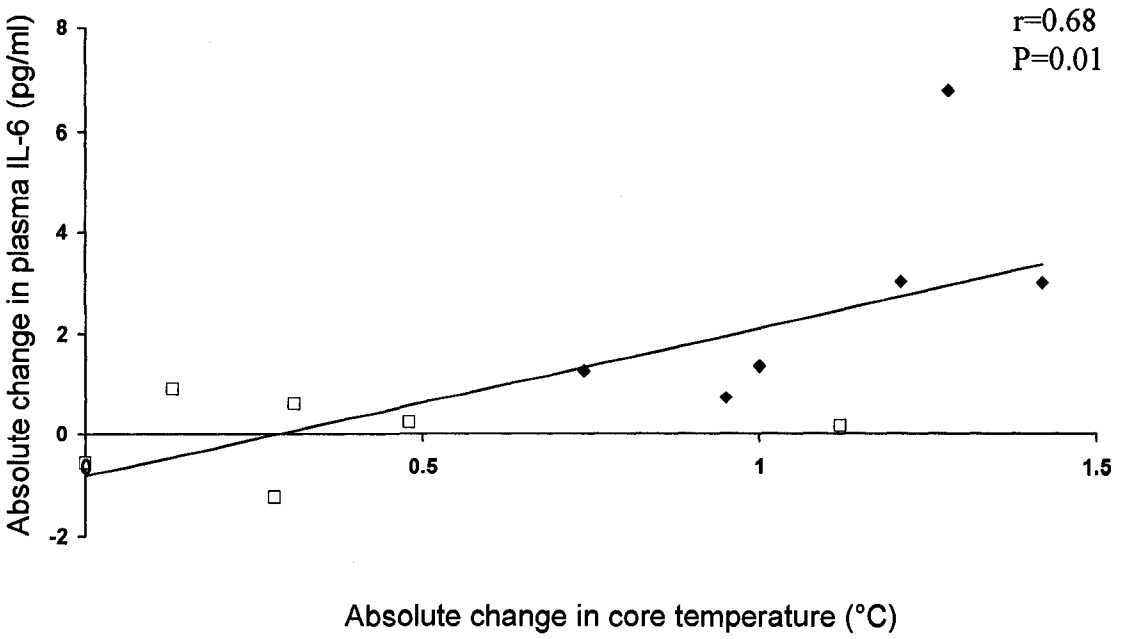

Figure 3.5: Absolute changes in concentration of plasma IL-6 in relation to absolute change in core body temperature in patients during the two heat exposure trials. Closed diamonds: subjects from higher intensity Experiment 1 (3 hours of heat exposure, 1.1±0.01°C core temperature increase). Open squares: lower intensity Experiment 2 (2 hours of heat exposure, 0.4±0.02°C core temperature increase). Significant Pearson's correlation: r=0.68, p=0.01.

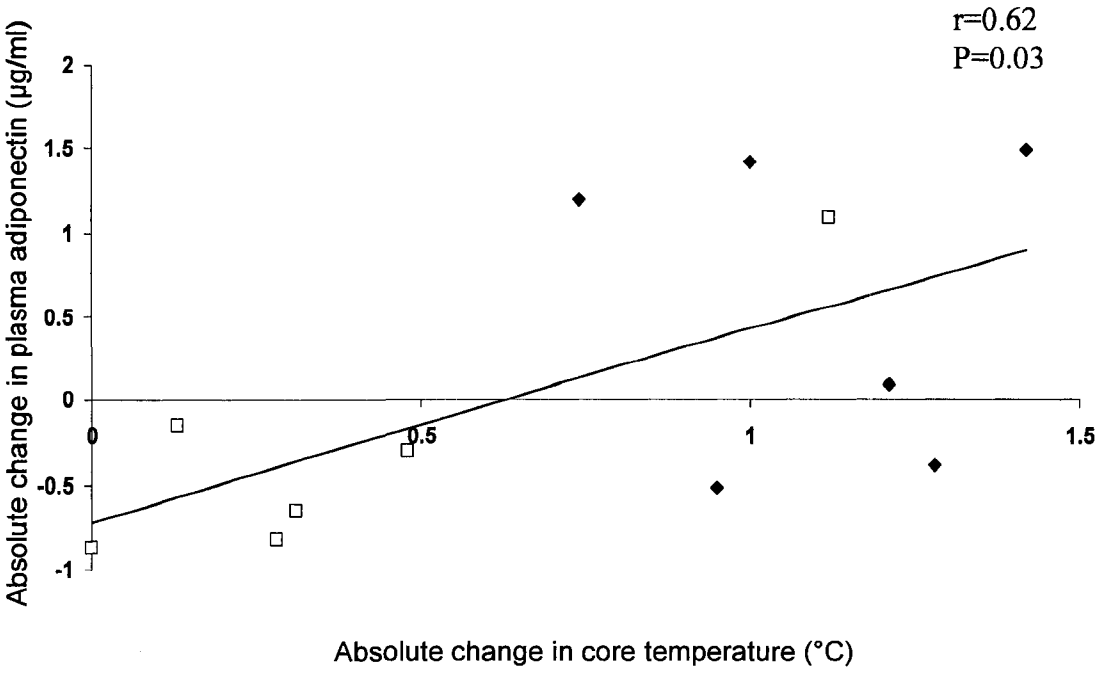

Figure 3.6: Absolute changes in concentration of plasma adiponectin in relation to absolute change in core body temperature in patients during two heat exposure trials. Closed diamonds: subjects from higher intensity Experiment 1 (3 hours of heat exposure, 1.1±0.01°C core temperature increase). Open squares: lower intensity Experiment 2 (2 hours of heat exposure, 0.4±0.02°C core temperature increase). Significant Pearson's correlation: r=0.62, p=0.03.

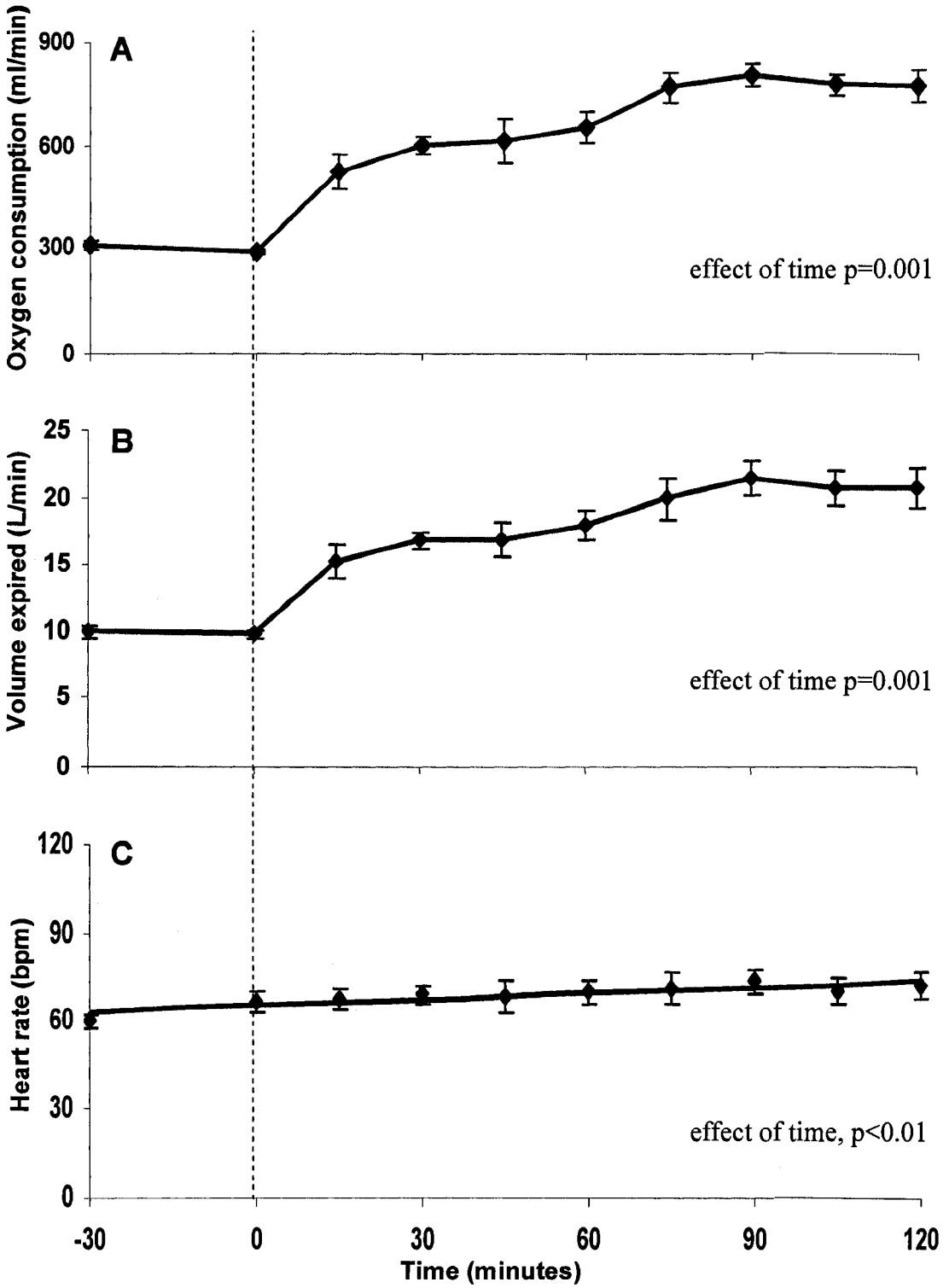

Figure 3.7: Changes in total energy expenditure (A), VE (B) and heart rate (C) over time during 2 hours of cold exposure with liquid perfusion suit (Experiment 3). Significant effect of time: energy expenditure ($p=0.001$), VE ($p=0.001$), heart rate ($p<0.01$).

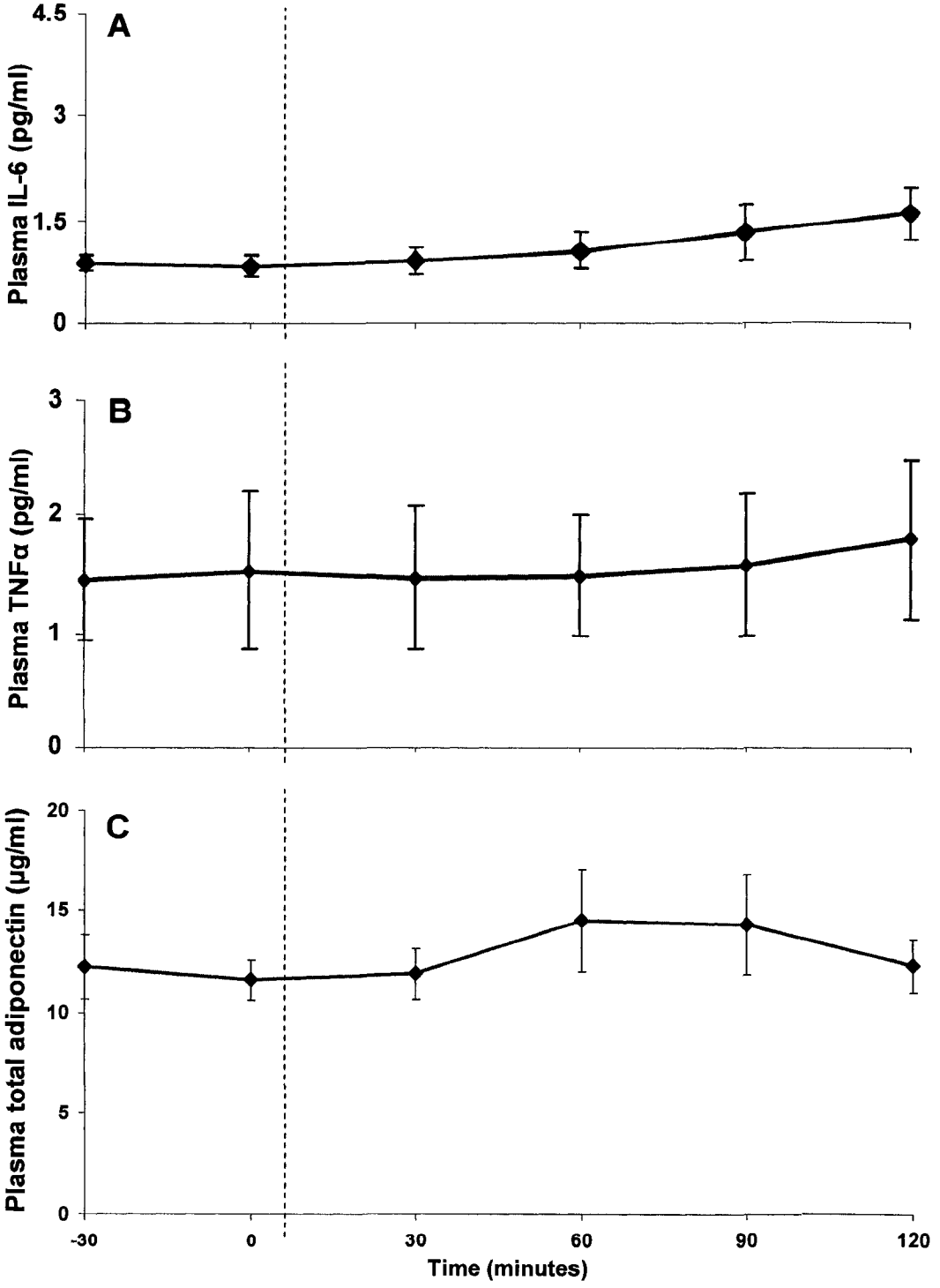

Figure 3.8: Changes in plasma IL-6 (A), TNFα (B) and total adiponectin (C) over time during 2 hours of cold exposure with liquid perfusion suit (Experiment 3). Plasma IL-6 and adiponectin levels increased over time but this fell short of statistical significance (p=0.065 and p=0.09 for IL-6 and adiponectin respectively).

Chapter 4 – Discussion

The purpose of this thesis was to examine changes in inflammatory levels in response to acute temperature stress. Experiments were performed using heat stress and cold stress. We hypothesized that both temperature stresses would trigger a response at the level of the inflammatory factors measured in our subjects' plasma. However, we anticipated different responses to the different stresses: with heat exposure hypothesized to promote a pro-inflammatory response and cold exposure hypothesized to create an anti-inflammatory response. Results showed that, during heat exposure, plasma IL-6 levels increased during the first heat exposure study (Experiment 1: 3 hours in a heated chamber) but not during the second study (Experiment 2: 2 hours in the liquid-conditioning suit). During the cold exposure in Experiment 3, plasma IL-6 and adiponectin concentrations increased over time but fell short of statistical significance. The inflammatory effects of each temperature stress will be further discussed in a separate section below.

Heat stress and inflammatory response

. This thesis has the advantage of using two different heating intensities to examine changes in inflammatory factors. Both heating intensities used in this thesis are lower than the heat stresses used in the human studies found in the above literature review. Experiment 1 achieved a greater heat intensity than Experiment 2: A one-way ANOVA was performed comparing the changes from baseline to final levels of core temperature, volume exhaled, heart rate and VE between the Experiments 1 and 2: the increase in core temperature was significantly greater in Experiment 1 ($p=0.005$).

A significant increase in plasma IL-6 was observed in Experiment 1. Plasma IL-6 is traditionally considered to be a pro-inflammatory factor representative of whole body

inflammatory status. This increase in plasma IL-6 was not observed during the lower intensity heat exposure of Experiment 2. In the human studies reviewed in Chapter 1, which used higher levels of temperature stress than in this thesis, plasma IL-6 was consistently shown to increase (Atanackovic et al. 2002; Bouchama et al. 1993; Hammami et al. 1997; Hashim et al. 1997; Robins et al. 1995). When the results from the two heat exposure trials were pooled together (shown in Figure 3.5), a significant positive correlation was found between changes in plasma IL-6 and changes in core temperature. This could suggest that plasma concentration of IL-6 may be directly affected by core temperature. Plasma IL-6 concentrations have been previously shown to be positively correlated with increase in core temperature (Hammami et al. 1997) and severity of illness in heat stroke patients (Bouchama et al. 1993). Our results suggest that the same holds true with lesser intensities of heat stress.

No change in plasma TNFα concentration was seen at either intensity of heat stress. In previous human studies, all of which used greater degrees of heat stress than were used in either Experiment 1 or 2, plasma TNFα concentrations increased in most (Atanackovic et al. 2002; Bouchama et al. 1991; D'Oleire et al. 1993; Robins et al. 1995), but not all studies (Hammami et al. 1997). Taken with the previous data on heat stress and plasma TNFα levels, the fact that we observed no change in TNFα concentration may be due to the lower intensity of heat stress used.

Here, plasma adiponectin was used as an anti-inflammatory reference point. During heat exposure, no significant change in adiponectin was seen over the course of either study. However, when the results from the two heat exposure trials were pooled together (shown in Figure 3.6), a significant positive correlation was found between

changes in plasma adiponectin and changes in core temperature. This could suggest that plasma concentration of adiponectin may be directly affected by core temperature. No previous work was found that directly assessed the effects of heat exposure either in humans, in animals or *in vitro*. To our knowledge, the only work dealing with heat-related parameters and adiponectin was a study in which plasma adiponectin levels increased when HSP72 (a heat shock protein induced by heat exposure which has been shown to have both anti-inflammatory and metabolic properties) was overexpressed in mice (Chung et al. 2008). This previous finding suggests that heat stress, as an inducer of HSP72, could increase the release of adiponectin. The positive correlation we found between changes in core temperature and changes in plasma adiponectin levels supports the notion that a factor associated with heat stress could mediate an increase in plasma adiponectin.

Besides heating duration and heating intensity, another potential important difference between Experiments 1 and 2 is the heating method used. Though both methods provide whole body heating through the convectional transfer of heat from an area of higher temperature (either the heated environment of the chamber or the heated water in the liquid conditioning suit), the body may respond differently to the two methods. Direct contact of the hot water-perfused liquid conditioning suit may cause a different inflammatory response in comparison to the heating chamber. During a preliminary trial with the liquid perfusion suit, red marks were noted on the trial subject in areas where the water-perfused tubing was pressed against the skin (such as back and back of legs when seated), suggesting a local inflammatory response. After this was

noted, in order to prevent any inflammatory effects of direct contact of the suit against skin and for comfort, subjects wore a t-shirt and spandex running pants under the suit.

Cold stress and inflammatory response

Plasma IL-6 concentrations showed a tendency to increase over time during the two hours of cold stress in Experiment 3. Repeated cold exposure in healthy young subjects has been shown to elevate plasma IL-6 levels in Scandinavian winter swimmers (Dugue and Leppanen. 2000) and following six weeks of hour-long immersions three times per week in 14°C water (Jansky et al. 1996). The acute effect of cold exposure in this group is less clear. The other human studies summarized in Chapter 1 assessed cold exposure combined with other stresses such as exercise, illness or surgery which could also stimulate an increase in plasma IL-6. These studies show a range of effects on plasma IL-6 concentrations (Aibiki et al. 1999a; Aibiki et al. 1999b; Grünenfelder et al. 2000; Hansen et al. 1999; Rasmussen et al. 2007; Rhind et al. 2001); it is not possible to separate the effects of cold stress from the effects of coexistent stresses. Our results are innovative in that they assess the acute effects of cold exposure without the presence of another stress on inflammation.

No change was seen in plasma concentrations of TNFα during cold exposure. Only one study without coexistent stresses summarized in Chapter 1 reports plasma TNFα levels, which tended to increase following repeated water immersions (Jansky et al. 1996). Like with IL-6, the combination of other stresses and cold exposure make other results difficult to interpret in terms of attempting to isolate the effects of cold exposure on TNFα. However, all the studies assessing changes in TNFα during cold exposure in humans showed either no change or tendencies that do not reach statistical

significance (Grünenfelder et al. 2000; Hansen et al. 1999; Jansky et al. 1996). These results along with our results showing no change in plasma TNFα concentrations suggest that plasma TNFα levels may not respond to cold exposure.

Plasma adiponectin, an anti-inflammatory factor released exclusively from adipocytes, had a tendency to increase with cold stress in this thesis. Although statistical significance was not reached here, previous work from our lab has shown a significanr increase in plasma adiponectin levels following cold exposure (Haman and Imbeault. 2005; Imbeault and Haman. 2007). Work assessing the effect of cold exposure on circulating adiponectin levels in rodents has varied with results showing an increase (Yoda et al. 2001), a decrease (Imai et al. 2006) or no effect (Puerta et al. 2002). Though results of the effect of cold exposure on circulating adiponectin levels in humans have been more consistent than in rodents, the cause of an increase is unclear.

Aside from the previous work from our group that has looked at changes of adiponectin during cold exposure (Haman and Imbeault. 2005; Imbeault and Haman. 2007), this is the first work that has looked at inflammation during cold exposure with liquid perfusion suits (as opposed to cold air, water immersion or ice packs). This different cooling method used may lead to different effects in response to cooling as water immersion may induce stress responses to both cold and wet and cooled air does not have the direct contact with skin that the liquid-perfused suit permits.

Chapter 5 – Perspectives, future directions and conclusions

Summary and perspectives

The aim of this thesis was to study the effects of heat and cold stresses on inflammatory response. Inflammation was measured through plasma concentrations of two of the most commonly measured pro-inflammatory cytokines, IL-6 and TNFα, which are accepted in the literature as being representative of the body's overall level of inflammation as well as through plasma levels of adiponectin, a protein specific to adipose tissue and known to have anti-inflammatory effects.

A summary of the findings from this thesis are shown in Figure 5.1. The only significant change in inflammation was seen in terms of an increase in plasma IL-6 during the higher intensity heat exposure of Experiment 1 (3 hours, heated chamber) and in terms of positive correlations which were found between increases in plasma levels of both IL-6 and adiponectin and increases in core body temperature during heat exposure.

Inflammatory and immune stress responses are thought to be triggered both by activation of the sympathetic nervous system as well as by activation of the hypothalamic-pituitary axis (Walsh and Whitham. 2006). In terms of physiological parameters (oxygen consumption, VE and heart rate), a significant increase in oxygen consumption and heart rate was seen in all three conditions and a significant increase in VE was seen with the higher intensity heat exposure (Experiment 1) and with cold exposure (Experiment 3). These physiological changes all suggest increased sympathetic nervous system activity, consistent with the expected response to a stress.

Although a common central pathway may be involved in the activation of inflammatory response to both heat and cold stress, it is possible that, although similarly activated in the two conditions, specific organs or cells respond differently to the

Table 5.1: Summary of major findings.

	Adiponectin	IL-6	TNFα
Heat stress:			
High intensity (Experiment 1)	-	↑	-
Lower intensity (Experiment 2)	-	-	-
Cold stress (Experiment 3)	Tended to ↑	Tended to ↑	-

activation depending on local environmental factors such as temperature. The presence of a Q10 effect (increased enzyme activity in relation to increased temperature) might be suggested during heat exposure by the correlations of the changes in plasma IL-6 and adiponectin levels with the changes in core temperature shown in Figures 3.5 and 3.6. The different effect of different environmental trigger might be suggested by the fact that in the in vivo human studies which isolate white blood cells from blood samples of subjects having undergone temperature stress, heat stress appears to induce the production of pro-inflammatory cytokines (Atanackovic et al. 2002; Downing and Taylor. 1987; Kappel et al. 1991b), whereas cold stress appears to reduce levels or release of such cytokines (Beilin et al. 1998; Rhind et al. 2001).

Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand–activated transcription factor associated mainly with adipose tissue but also found elsewhere in the body (Boon Yin et al. 2008; Sharma and Staels. 2007). It is involved in both adipokine and inflammatory factor production (Ahmed et al. 2007). PPARγ agonists have been shown to stimulate adiponectin release (Sharabi et al. 2007). The possibility that PPARγ is more activated in the heat due to the Q10 effect is quite possible. In addition, our research has shown an increased plasma non-esterified fatty acid (NEFA) concentration and an increased degree of NEFA unsaturation in plasma NEFA of human subjects following 3 hours of heat exposure (Dickson et al, article in progress). Fatty acids have been shown to act as ligands stimulating PPARγ. Different fatty acids have different affinities for binding with PPARγ: unsaturated fatty acids have been shown to be stronger ligands (Kliewer et al. 1997). Therefore, the increase in degree of unsaturation seen during heat exposure may activate PPARγ, stimulating the release of adiponectin.

Future directions

As described in Chapter 1, IL-6 is released from several sources in the body: some of these sources contribute mainly to local IL-6 levels whereas others contribute more to systemic levels reflected by plasma IL-6. Macrophages and other white blood cells are the main source of IL-6. Adipose tissue and skeletal muscle have also been identified as contributors to systemic levels of IL-6. Plasma concentrations measured in this thesis do not divulge information regarding the source of the increase in plasma IL-6 seen in the higher heat intensity (Experiment 1). However, possible sources can be suggested: Studies measuring pro-inflammatory factors in white blood cells isolated from the blood samples of heat-exposured human subjects showed increased levels of inflammatory factors (Atanackovic et al. 2002; Downing and Taylor. 1987; Park et al. 1990), suggesting a role for white blood cell lines in the elevation of plasma IL-6 seen following heat exposure in Experiment 1. Based on the acute elevation of body temperature associated with exercise, we may tentatively choose to examine the research on sources of acute inflammatory factor release in exercise; this may shed some light on the potential sources of elevated plasma IL-6 seen during heat exposure in Experiment 1. Despite suggestions that over the long term, exercise has anti-inflammatory effects (Dekker et al. 2007; Goldhammer et al. 2005; Oberbach et al. 2006; Walther et al. 2008), acutely, exercise has been shown to trigger the release of pro-inflammatory factors such as IL-6 from white blood cells (Peake et al. 2008), muscle (Keller et al. 2001; Pedersen and Fischer. 2007a; Pedersen and Fischer. 2007b) and subcutaneous abdominal adipose tissue (Hojbjerre et al. 2007). In the cold, though only a trend towards an increase in plasma IL-6 was seen, changes at the level of IL-6 release from different sources in the body may

occur. As mentioned above, contracting muscle appears to be a source of IL-6 release (Al-Khalili et al. 2006; Pedersen and Fischer. 2007b; Ruderman et al. 2006; Steensberg et al. 2003; Weigert et al. 2005). Moderate intensity shivering was induced during the cold exposure session and, thus, skeletal muscle contractions during shivering could be a likely source of increased IL-6 release in the cold. *In vitro* work has shown delayed but increased IL-6 release from LPS-stimulated monocytes in the cold (Fairchild et al. 2000; Matsui et al. 2006), suggesting that monocyte and macrophage IL-6 release may be modified with cold exposure.

A potential follow-up of the work from this thesis would be a repeat of the protocols of Experiments 1 and 3, taking muscle and fat biopsies before and after temperature stress and isolating macrophages from pre and post blood samples. Reverse-transcription polymerase chain reaction (RT-PCR) could then be used to measure messenger ribonucleic acid (mRNA) levels of IL-6 which could be compared between pre and post samples from the three sources to determine if changes in tissue-specific IL-6 production occurred. mRNA levels of PPARγ could also be measured from these biopsy samples in order to assess the effect of temperature stress on this transcription factor which is known to be involved in adipokine production, inflammation and fat metabolism. An alternative to biopsy work would be *in vitro* work assessing adipocytes' and myocytes' inflammatory responses to heat and cold stresses. Neither of these tissues has been assessed during temperature stress and *in vitro* work of this nature would enable us to isolate cell-specific responses to temperature stress.

Unlike many of the human and animal temperature studies described in Chapter 1, the subjects in all experiments that were part of this thesis were young, healthy and not

exposed to coexistent illnesses or stresses. Young healthy subjects are likely to respond differently to heat/cold than subjects with pre-existing medical conditions (cancer, stroke, head injuries, heat disease). This could be due in part to either under or over-active immune systems seen with various co-existent diseased or stressed states. While some co-existing medical conditions have been looked at during temperature stress, little has been done in obese and diabetic population. The diabetic and obese populations are known to respond differently to temperature stress (Zahorska-Markiewicz. 1982) and to have a mildly elevated baseline level of inflammation (Maachi et al. 2004; Malavazos et al. 2007; Park et al. 2005; Pou et al. 2007). This baseline level of inflammation is thought to be responsible for at least some degree of insulin resistance or impaired fat oxidation seen in these groups (Pradhan et al. 2001; Rubin et al. 2008; Tilg and Moschen. 2008; Xu et al. 2003). It is possible that inflammatory factors in these groups may respond differently to temperature stress. The only study found during our literature review assessing diabetic or obese subjects during temperature stress was a small study which has suggested potential beneficial effects of heat stress in blood sugar control (Hooper. 1999). Work done with obese and diabetic populations using temperature stress should thus address potential changes in insulin resistance.

Conclusion

This thesis demonstrates a heat-induced increase in plasma IL-6 and a relationship between increase in core temperature and increase in both IL-6 and adiponectin. A trend was also seen towards increases in both IL-6 and adiponectin during cold exposure, though statistical significance was not reached. The novelty of this work lies in the use of two different heat exposure intensities, both lower than the majority of heating intensities

used in previous literature and the quantification of adiponectin levels in human subjects during temperature stress. Further exploring these changes with biopsy studies and in obese and diabetic populations will help us to further understand how inflammatory factors can be modified, where these changes occur and how they changes differ between healthy lean subjects and obese or diabetic subjects.

Appendix A

Physical Activity and Food Intake Questionnaire

Date:

Subject ID:

Trial Number:

Trial Condition:

Weight:

1-) At what time did you last eat?

- Did you eat your assigned frozen dinner last night?

2-) Did you take in any caffeine in the last 48 hours (coffee, tea, cola...)?

- If yes, how many servings?
- When was your last one?

3-) How many alcoholic beverages did you have in the past 48 hours?

- When was your last one?

4-) Please fill in the physical activity chart on the next page. You are asked to classify the intensity of any activities you did for every half hour period over the past 24 hours. Please refer for the coding chart for activity categories.

Date:

Subject ID:

Physical Activity Table

	1	2	3	4	5	6	7	8
7:30-8:00								
8:00-8:30								
8:30-9:00								
9:30-10:00								
10:00-10:30								
10:30-11:00								
11:00-11:30								
11:30-12:00								
12:00-12:30								
12:30-13:00								
13:00-13:30								
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2:30-3:00								
3:00-3:30								
3:30-4:00								
4:00-4:30								
4:30-5:00								
5:00-5:30								

5:30-6:00								
6:00-6:30								
6:30-7:00								
7:00-7:30								

Adapted from:

24 Hour Physical Activity Recall Questionnaire of Kuopio Ischemic Heart Disease Study (KIHD) (Salonen and Lakka. 1987)

Bouchard 3-Day Physical Activity Questionnaire (Bouchard et al. 1983)

Category of activity	Example of activity for each category	Approximate energy expenditure (kcal/kg/15 min)
1	Lying down: - sleeping - resting in bed	0.26
2	Seated: - listening in class - eating - writing by hand or typing - reading - listening to the radio or T.V. - taking a bath	0.38
3	Standing; light activity: - washing oneself - shaving - combing hair - dusting - cooking	0.57
4	Getting dressed Taking a shower Driving a car Taking a walk (strolling)	0.70
5	Light manual work: - housework (washing windows, sweeping etc.) - tailor - baker - printer - brewer - cobbler - mechanic - electrician - painter - lab-work - carpentry - masonry - driving a farm tractor - cleaning trees - working in the chemical or electric industries - feeding animals on a farm - doing the bed Riding a moped Moderately quick walking (going to school, shopping)	0.83
6	Light sport or leisure activities: - light canoeing - volleyball - table tennis - baseball (except the pitcher) - golf - rowing - archery - ninepins - croquet - sailing - cycling (leisure)	1.20
7	Moderate manual work: - machine operating (building industry) - repairing a fence - loading bags or boxes - plantation work - forest work (machine sawing and log handling) - mine work - shoveling snow	1.40
8	Moderate sport or leisure activities: - baseball (pitcher) - badminton - canoeing - cycling (race bike) - dancing - tennis - jogging (slow running) - horseback riding - Alpine skiing - cross-country skiing (leisure) - swimming - gymnastics - brisk walking	1.0

From: Bouchard 3-Day Physical Activity Questionnaire (Bouchard et al. 1983)
*Note: approximate energy expenditure for category 8 corrected to 1.60.

Appendix B

Physical activity levels of day prior to study for subjects of second heat exposure study (Experiment 2)												
	Category	1	2	3	4	5	6	7	8			
	Approximate energy expenditure per 30min (kcal/kg)	0.52	0.76	1.14	1.4	1.66	2.4	2.8	3.2			
Subject										Sum	Body weight (kg)	Approximate energy expenditure (kcal)
1	Number of time blocks / category	16	9	12	5	5	0	0	0			
	Energy expenditure (kcal/kg)	8.32	6.84	13.68	7	8.3	0	0	0	44.14	71.58	3159.541
2	Number of time blocks / category	15	3	3	8	2	0	0	16			
	Energy expenditure (kcal/kg)	7.8	2.28	3.42	11.2	3.32	0	0	51.2	79.22	83.6	6622.792
3	Number of time blocks / category	19	11	13	1	3	0	0	0			
	Energy expenditure (kcal/kg)	9.88	8.36	14.82	1.4	4.98	0	0	0	39.44	79.02	3116.549
4	Number of time blocks / category	16	12	2	4	13	0	0	0			
	Energy expenditure (kcal/kg)	8.32	9.12	2.28	5.6	21.58	0	0	0	46.9	82.55	3871.595
5	Number of time blocks / category	17	11	0	10	0	0	0	9			
	Energy expenditure (kcal/kg)	8.84	8.36	0	14	0	0	0	28.8	60	91.99	5519.4
6	Number of time blocks / category	19	14	3	1	4	0	0	6			
	Energy expenditure (kcal/kg)	9.88	10.64	3.42	1.4	6.64	0	0	19.2	51.18	83.73	4285.301

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